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**LASER CAPTURE MICRODISSECTION
TRANSLATION STAGE JOYSTICK**

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application is, under 35 U.S.C. § 120, a continuation of U.S.
5 Ser. No. 09/018,452, filed February 4, 1998, now pending, which is in-turn
a continuation-in-part of both U.S. Ser. No. 60/060,731, filed October 1,
1997, now pending, and U.S. Ser. No. 60/037,864, filed February 7, 1997,
now abandoned, the entire contents of all which are hereby incorporated
herein by reference as if fully set forth herein.

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BACKGROUND OF THE INVENTION

Field of the Invention

15 The invention relates generally to the field of laser capture
microdissection (LCM). More particularly, the invention relates to inverted
microscopes that include specialized apparatus for performing LCM.
Specifically, a preferred implementation of the invention relates to an
inverted microscope that includes a cap handling subsystem, an
20 illumination/laser optical subsystem, a vacuum chuck subsystem, and a

manual joystick subsystem. The invention thus relates to inverted microscopes of the type that can be termed laser capture microdissection inverted microscopes.

5 **Discussion of the Related Art**

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from
10 the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

15 A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture
microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene
20 vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

25 In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the

tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes
5 localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue
10 section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the
15 tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ
20 breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

25 Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research.

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For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

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The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

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A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

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LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

5 The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is
10 mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

15 While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

20 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for laser capture microdissection. There is also a particular need for an improved method of laser capture microdissection.

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A first aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection method, comprising: providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument; 10 providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface; placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and then transferring a portion of said sample to said laser capture microdissection transfer film, 15 without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.

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A second aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection instrument, comprising: an inverted microscope including: an illumination/laser optical subsystem; a translation stage coupled to said illuminator/laser optical subsystem; a cap handling subsystem coupled to said translation stage; a vacuum chuck subsystem coupled to said translation stage; and a manual joystick subsystem coupled to said translation stage.

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These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

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BRIEF DESCRIPTION OF THE DRAWINGS

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A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

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FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention;

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1;

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

5 FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention;

10 FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention;

FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6;

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention;

15 FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8;

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention;

20 FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10;

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention;

25 FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention;

FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention;

5 FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention;

FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention;

10 FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention; and

FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

15 **DESCRIPTION OF PREFERRED EMBODIMENTS**

The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed 20 in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

25 The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997 entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed

October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as

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polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

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The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement. Further, this simultaneous movement can be effected with in a single handed manner. The acquisition of samples is thus made quicker.

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Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a function of operator hand movement.

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In addition, the joystick provides tactile feedback not available with electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X

and Y) as a function of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

5 Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185. After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.

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20 Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be appreciated from FIG. 2B that the illumination beam path includes delivery

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of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which can include a camera. In idle mode, the laser beam path 310 provides a visible low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second, preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength

of the laser is 810 nannometers. In a preferred embodiment the spot size of
the laser at the EVA material located on microcentrifuge tube cap 120 is
variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more
preferably from 5 to 30 microns. These ranges are relatively preferred when
5 designing the optical subsystem. From the standpoint of the clinical
operator, the widest spot size range is the most versatile. A lower end point
in the spot size range on the order of 5 microns is useful for transferring
single cells.

10 Suitable lasers can be selected from a wide power range. For
example, a 100 watt laser can be used. On the other hand, a 50 mW laser
can be used. The laser can be connected to the rest of the optical subsystem
with a fiber optical coupling. Smaller spot sizes are obtainable using
diffraction limited laser diodes and/or single mode fiber optics. Single
mode fiber allows a diffraction limited beam.

15 While the laser diode can be run in a standard mode such as TEM_{00} ,
other intensity profiles can be used for different types of applications.
Further, the beam diameter could be changed with a stepped lens instead of
lens 350.

20 Changing the beam diameter permits the size of the portion of the
sample that is acquired to be adjusted. Given a tightly focused initial
condition, the beam size can be increased by defocusing. Given a defocused
initial condition, the beam size can be decreased by focusing. The change in
focus can be in fixed amounts. The change in focus can be obtained by
means of indents on a movable lens mounting and/or by means of optical
25 glass steps. In any event, increasing/decreasing the optical path length is the
effect that is needed to alter the focus of the beam, thereby altering the spot

size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic), and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial. Fluorescence emitted by the sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for damping vertical motion of the cap handling mechanic subassembly 110.

5 The dampener 510 is adapted to lower the microcentrifuge tube cap 120 down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable of retarding the vertical motion of the subassembly 110 so as not to generate an impulse. As

10 the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before

15 the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to

20 microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube

25 cap 120 and then the microcentrifuge tube cap 120 is picked up off of the

slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is

lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the

5 microcentrifuge tube cap 120 off the slide (not shown).

The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture

10 microdissection transfer film is approximately 0.26 square inches.

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

15 Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the

20 microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to

25 the position depicted in FIGS. 6-7).

Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and

the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

5 The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place.

10 Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

15 Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

20 It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

25 Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430 collimates the light from the fiber optic 1410. The collimator lens 1430 can

be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens).
A collimated beam 1440 from the collimator lens 1430 then passes through a
beam splitter 1450. The beam splitter 1450 permits the injection of a laser
beam 1460. After reflection by the beam splitter 1450, the laser beam 1460
5 is coaxial with the white light illumination. Both types of light then reach a
condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG
010) or (01 LAG 010) or other similar aspheric-like lens. The condensed
coaxial beams are then incident upon and pass through the microcentrifuge
tube cap 120. The focusing beam that results from the condenser lens 1470
10 can have a numerical aperture of approximately 0.8. This can be
characterized as a focusing beam. The microcentrifuge tube cap 120 is
located on top of a slide with cells to be sampled (not shown).

15 Turning now to FIG. 15, another embodiment of the high numerical
aperture illuminator is depicted. In this embodiment, a diffuser 1500 is
located beneath the condenser lens 1470 at above the glass slide 130 that
contains the cells to be sampled.

20 More generally, any suitable scattering media can be used to provide
the functions of the diffuser 1500. Providing such a scattering media near
the tissue to scatter the light results in dramatically improved illumination of
the sample and much better visualization. A scattering media of this type
eliminates the need for refractive index matching of the sample. Such a
scattering media can allow visualization of the cell nucleus and other
subcellular structures that would normally be obscured by normal
illumination techniques.

25 The scattering media can be a diffuser material. A diffuser material
that is suitable for use as the scattering media is milk or opal glass which is

a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example, 5 frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser. In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light 10 sufficiently.

Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed 15 into the microcentrifuge tube cap 120 so as to acquire the desired sample.

The purpose of the illuminator design is to provide a very high numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization, 20 this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important 25 because the sample is adjacent the bottom of the sample carrier and the

objective cannot move closer to the sample than the top of the sample carrier.

The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in visualization. This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a

speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is

pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the velocity should be such that the sample is thixotropically sheared.

5

Practical Applications of the Invention

A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases.
10 This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient
15 samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually
20 innumerable uses for the invention, all of which need not be detailed here.

Advantages of the Invention

A laser capture microdissection instrument and/or method
25 representing an embodiment of the invention can be cost effective and advantageous for at least the following reasons. The invention will replace

current methods with better technology that allows for more accurate and reproducible results. The invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent was specifically and individually indicated to be incorporated in its entirety by reference.

All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the

LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

5

10

It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
 - providing a sample that is to undergo laser capture microdissection;
 - positioning said sample on a translation stage of a laser capture microdissection instrument and within an optical axis of said laser capture microdissection instrument;
 - providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface;
 - placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and then
 - moving said sample and said translation stage with a manual joystick subsystem that is connected to said translation stage; and then
 - transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.
2. The laser capture microdissection method of claim 1, wherein moving said sample and said translation stage with said manual joystick subsystem includes simultaneous X and Y movement.

3. The laser capture microdissection method of claim 1, wherein moving said sample and said translation stage with said manual joystick subsystem includes reducing a scalar movement defined by an operator.
4. A laser capture microdissection instrument, comprising:
 - a translation stage; and
 - a manual joystick subsystem coupled to said translation stage.
5. The laser capture microdissection instrument of claim 4, wherein said manual joystick subsystem includes a joystick that is coupled to said translation stage through a first spherical mounting that is movably connected to said joystick and a bracket that is mechanically connected to both said spherical mounting and said translation stage.
6. The laser capture microdissection instrument of claim 5, wherein said manual joystick subsystem includes a joystick having a second spherical mounting that is movably connected to a static bracket.
7. The laser capture microdissection instrument of claim 6, wherein a first length between said first spherical mounting and said second spherical mounting is less than a second length between said second spherical mounting and a bottom end of said joystick.
8. The laser capture microdissection instrument of claim 7, wherein a ratio of said first length to said second length is less than 1/5.

9. The laser capture microdissection instrument of claim 8, wherein said ratio is approximately 1/7.
10. The laser capture microdissection instrument of claim 4, further comprising an illumination/laser optical subsystem.
11. The laser capture microdissection instrument of claim 4, further comprising a transfer film carrier handling subsystem.
12. The laser capture microdissection instrument of claim 4, further comprising a vacuum chuck subsystem connected to said translation stage.
13. An inverted microscope, comprising:
a translation stage; and
a manual joystick subsystem connected to said translation stage.
14. The inverted microscope of claim 13, wherein said manual joystick subsystem includes a joystick that is coupled to said translation stage through a first spherical mounting that is movably connected to said joystick and a bracket that is mechanically connected to both said spherical mounting and said translation stage.
15. The inverted microscope of claim 14, wherein said manual joystick subsystem includes a joystick having a second spherical mounting that is movably connected to a static bracket.

16. The inverted microscope of claim 15, wherein a first length between said first spherical mounting and said second spherical mounting is less than a second length between said second spherical mounting and a bottom end of said joystick.
17. The inverted microscope of claim 16, wherein a ratio of said first length to said second length is less than 1/5.
18. The inverted microscope of claim 17, wherein said ratio is approximately 1/7.
19. The inverted microscope of claim 13, further comprising an illumination/laser optical subsystem.
20. The inverted microscope of claim 13, further comprising a transfer film carrier handling subsystem.
21. The inverted microscope of claim 13, further comprising a vacuum chuck subsystem connected to said translation stage.

ABSTRACT OF THE DISCLOSURE

Systems and methods for laser capture microdissection are disclosed.

An inverted microscope includes a translation stage joystick subsystem.

The systems and methods provide the advantages of increased speed and
much lower rates of contamination.

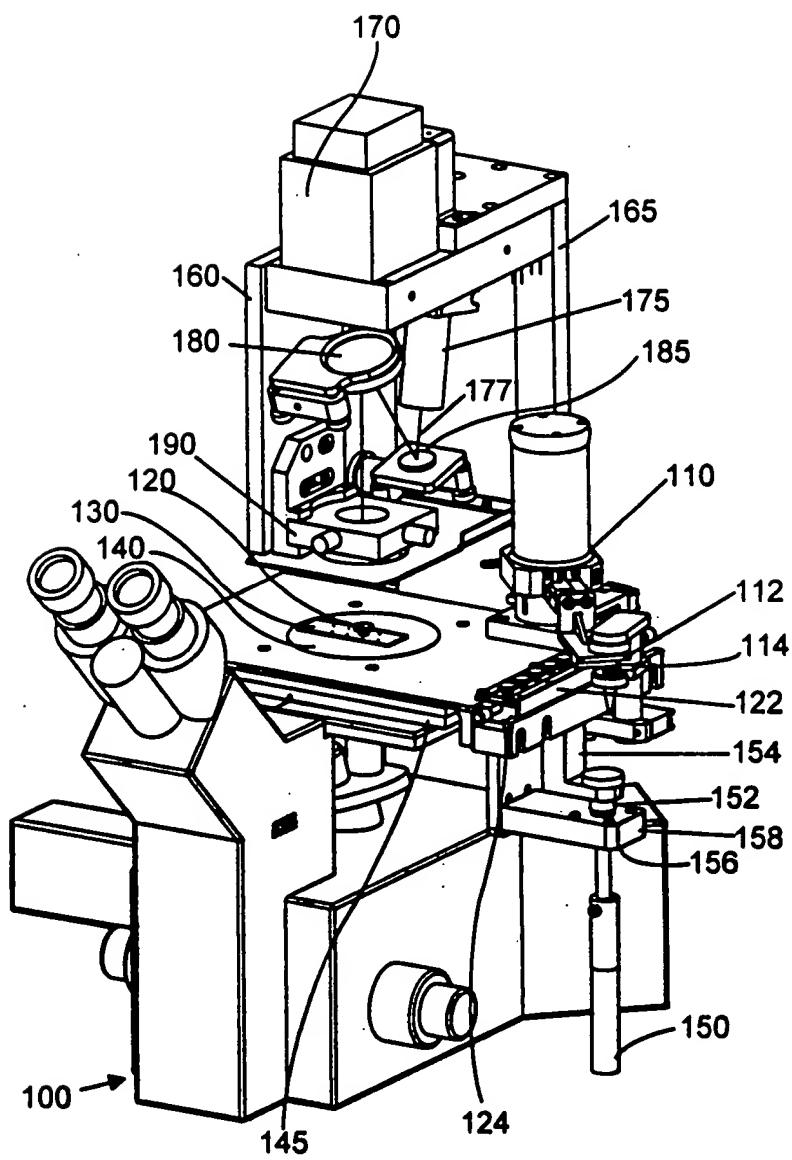
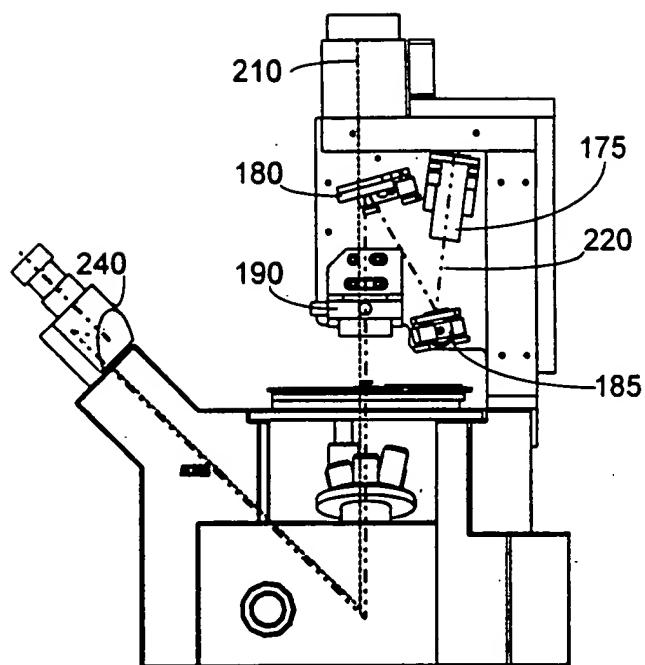
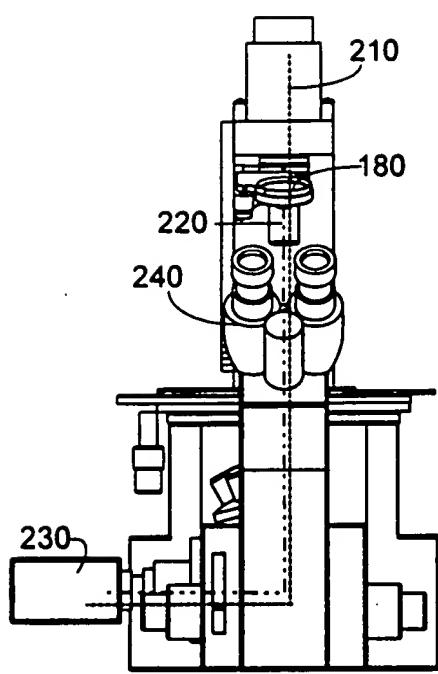


FIG. 1



— WHITE LIGHT ILLUMINATION PATH
— LASER BEAM PATH

FIG. 2A

FIG. 2B

FIG. 3

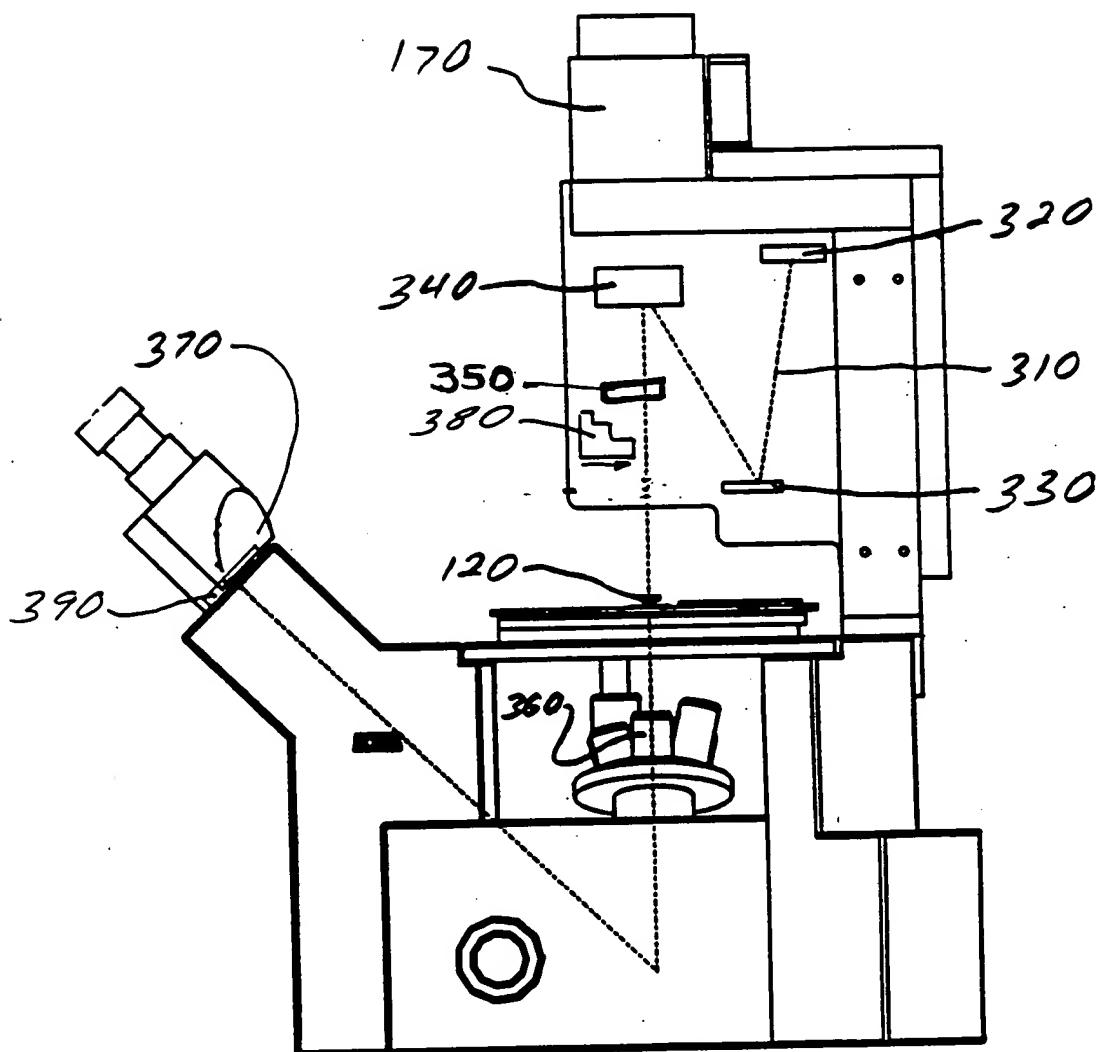
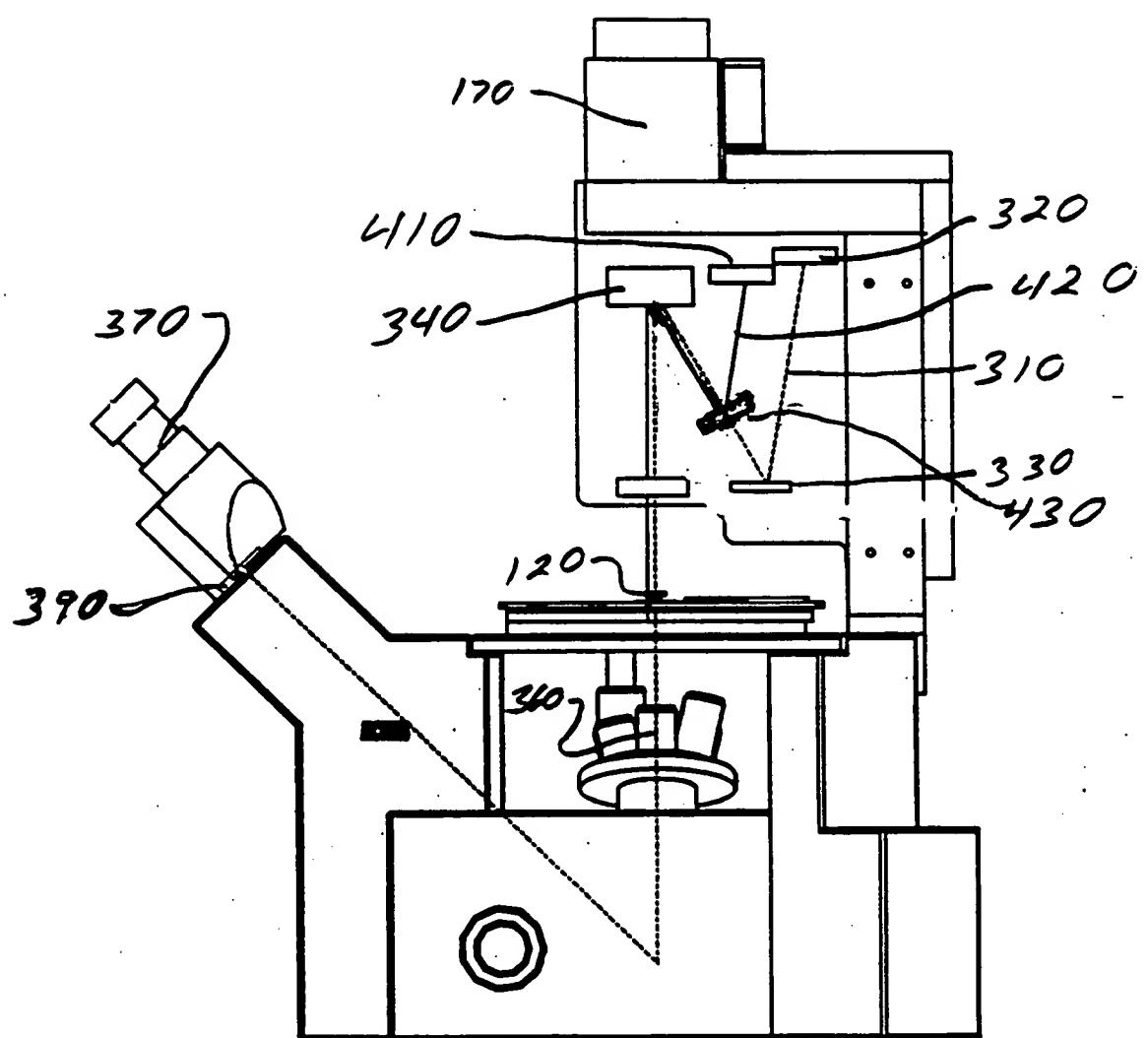


FIG. 4



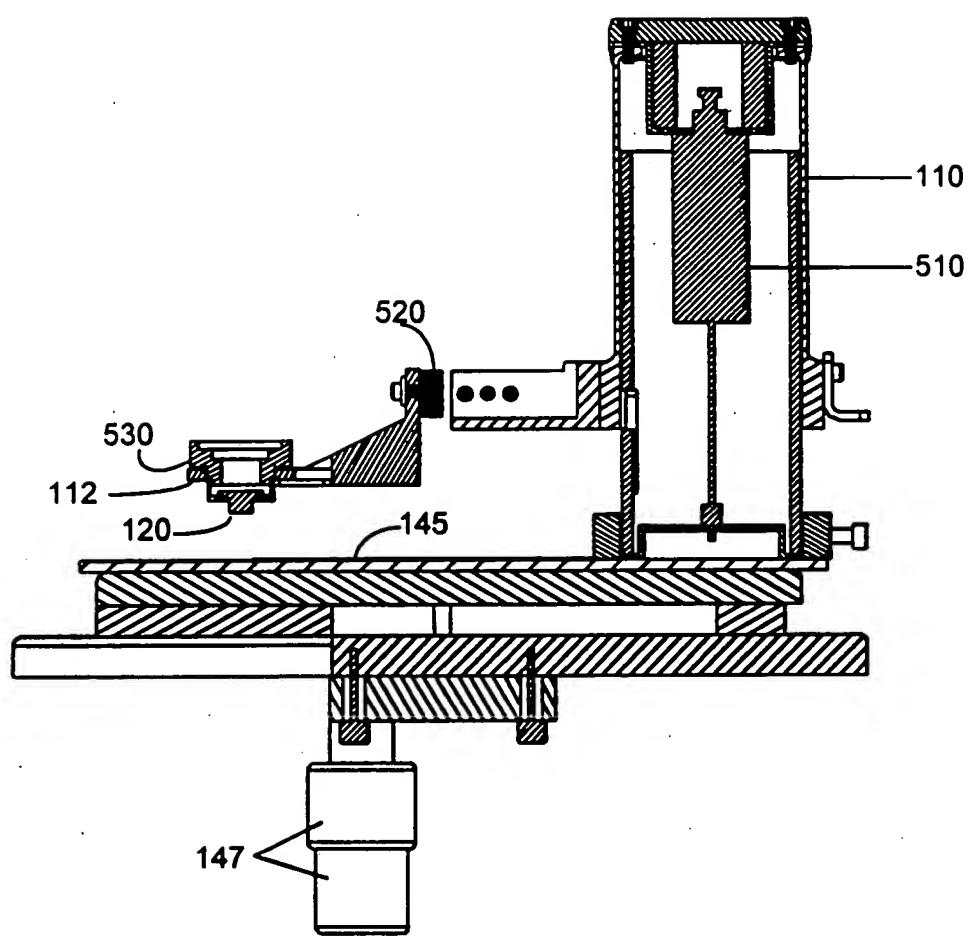


FIG. 5

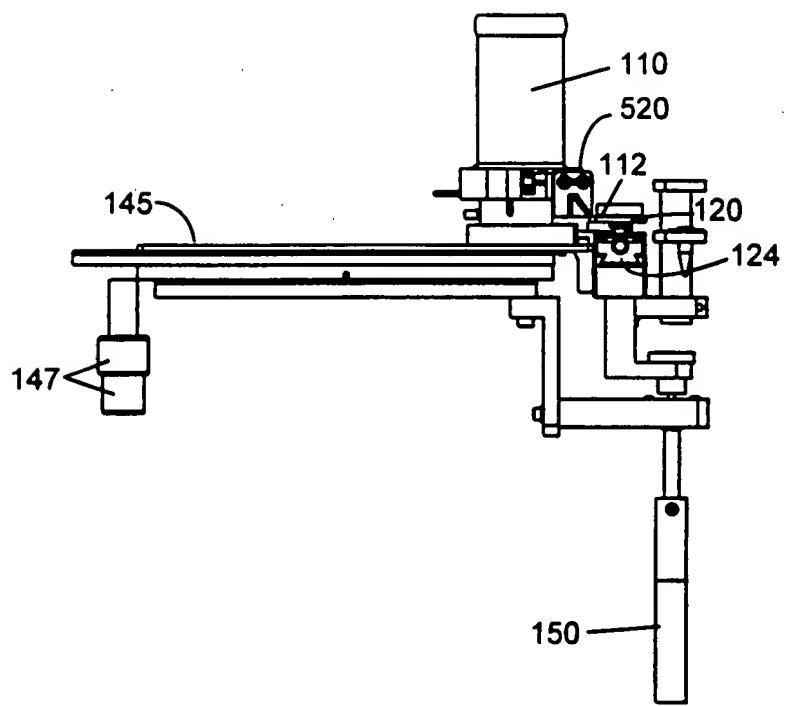


FIG. 6

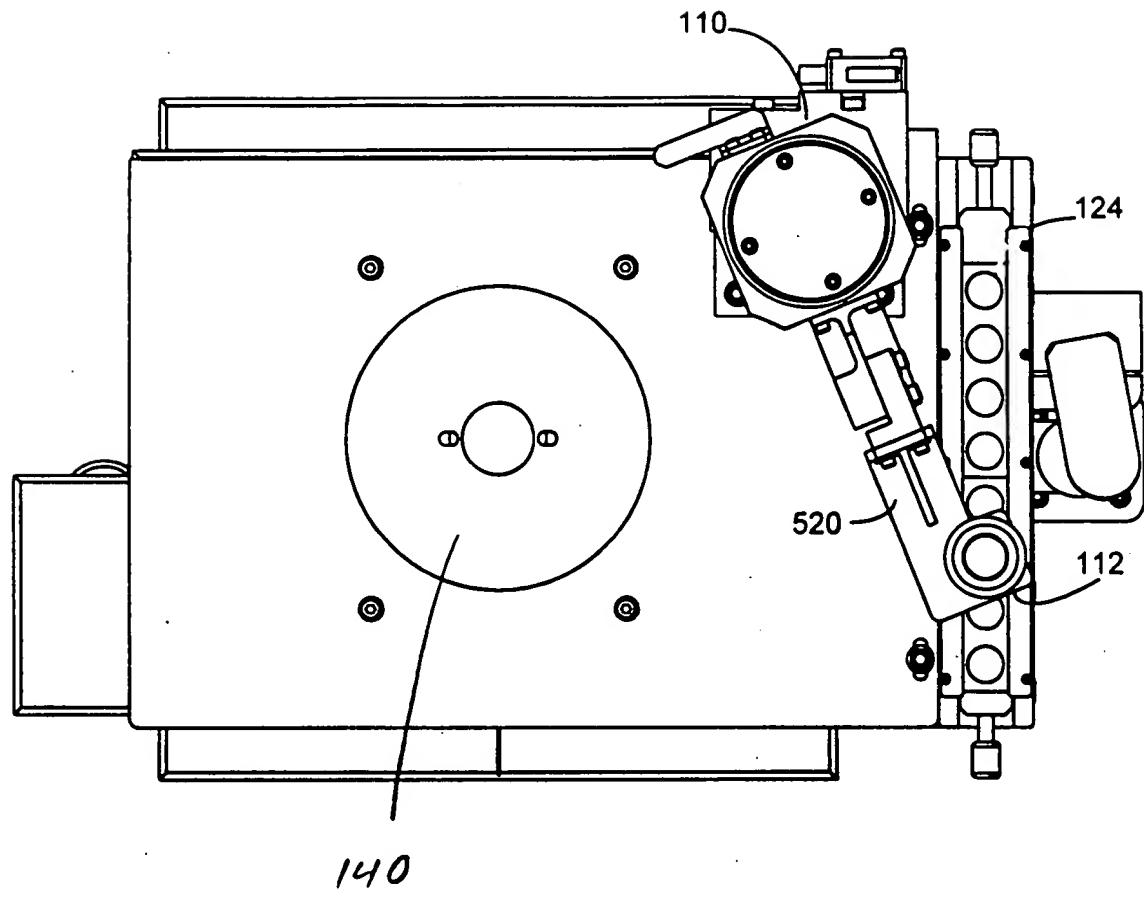


FIG. 7

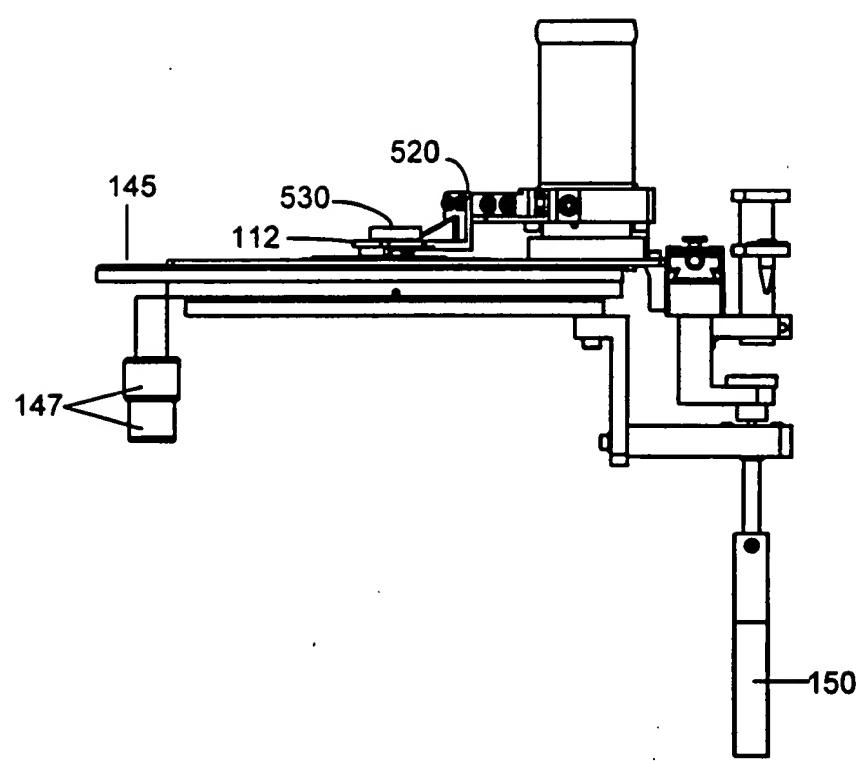


FIG. 8

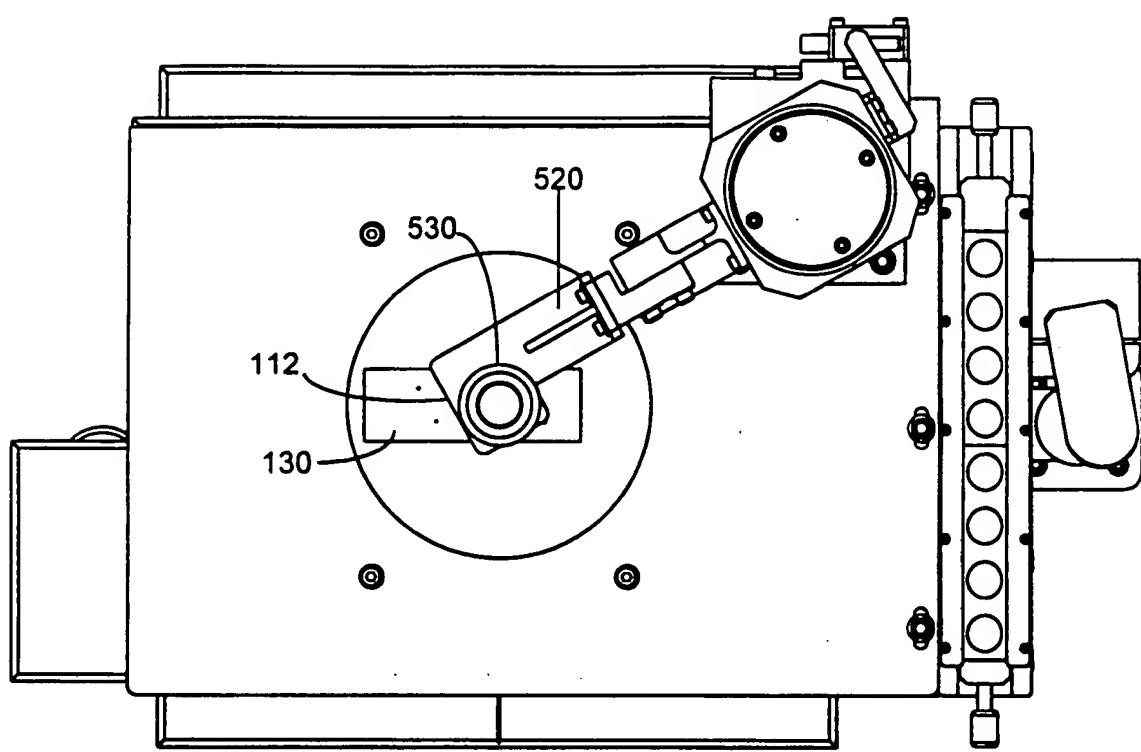


FIG. 9

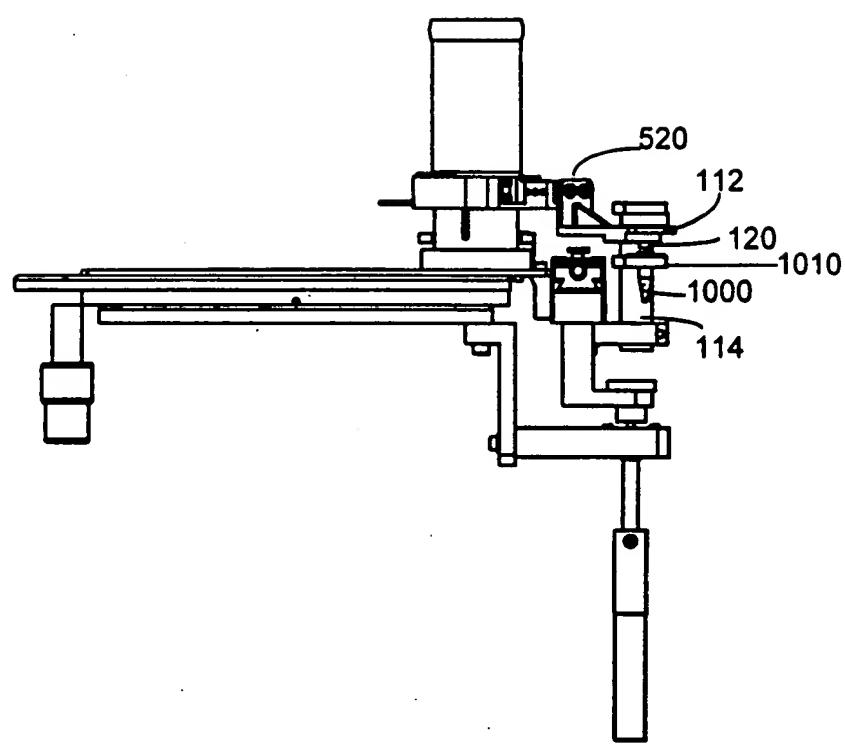


FIG. 10

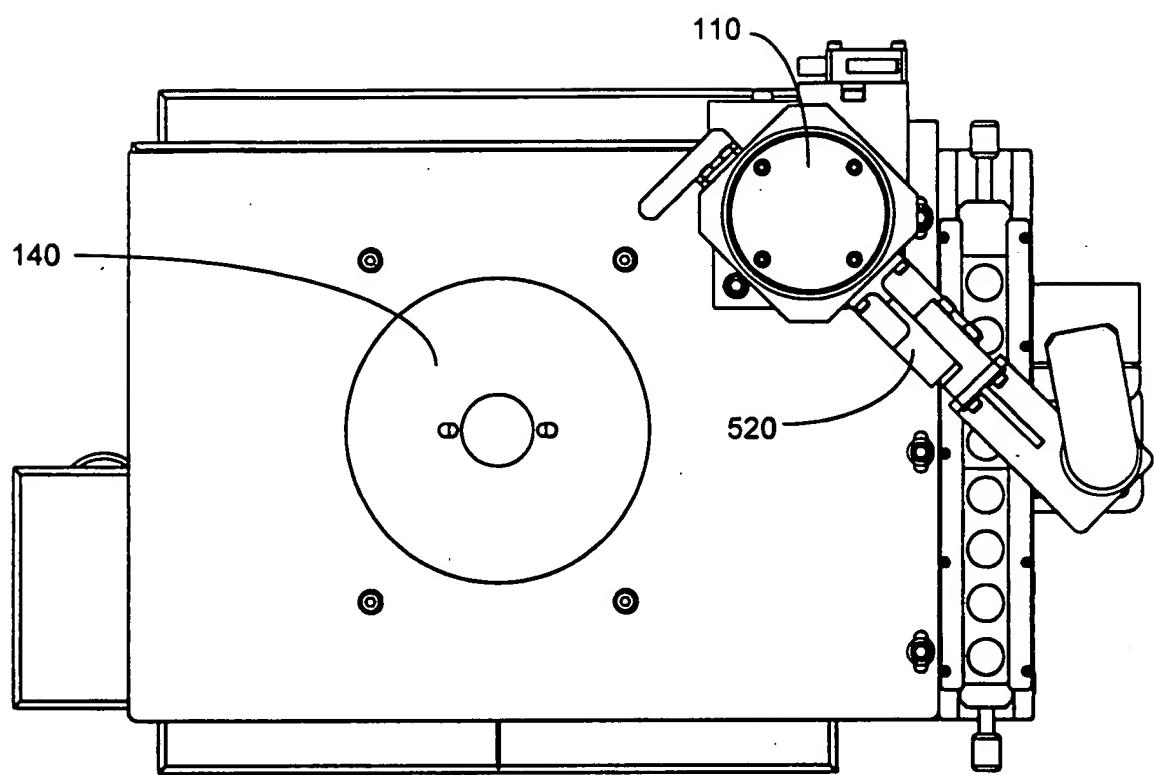


FIG. 11

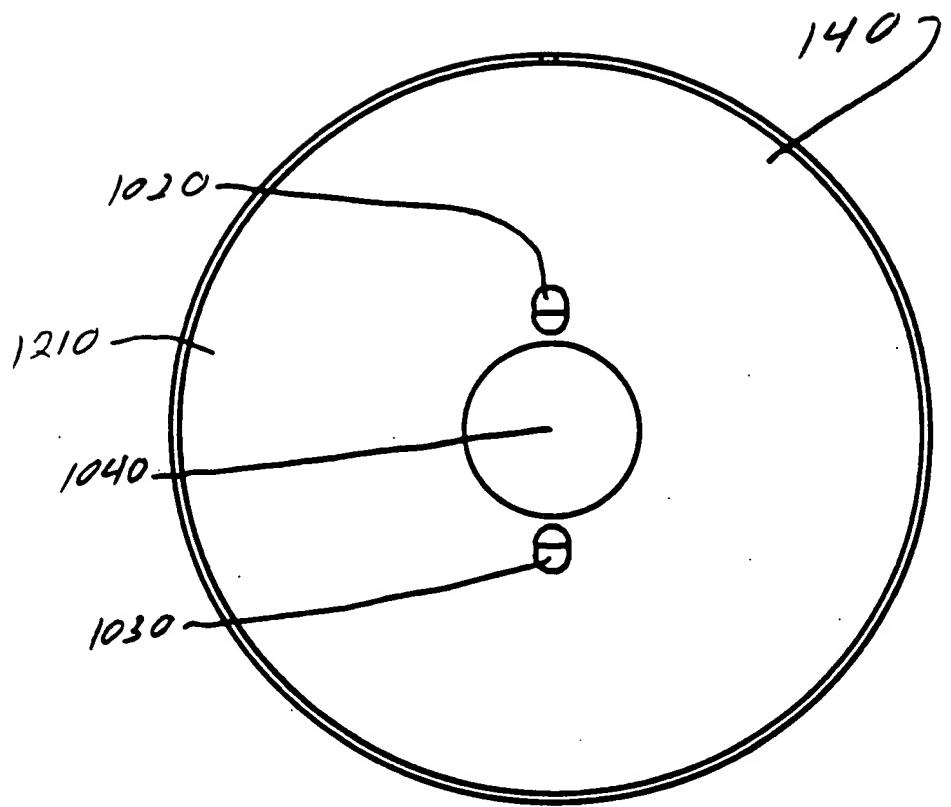


FIG. 12

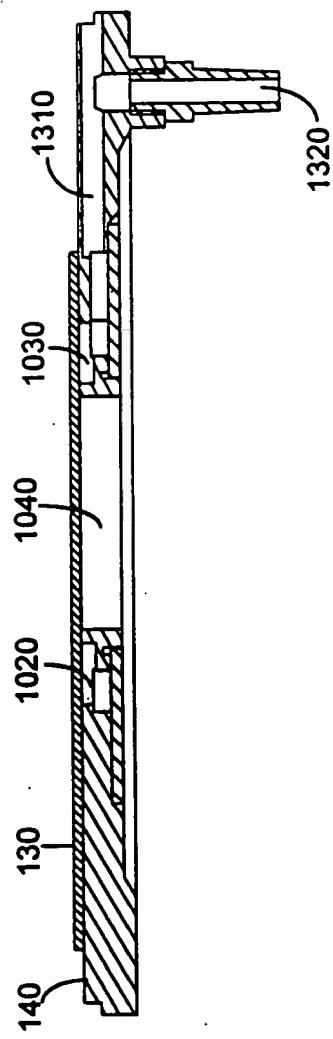


FIG. 13

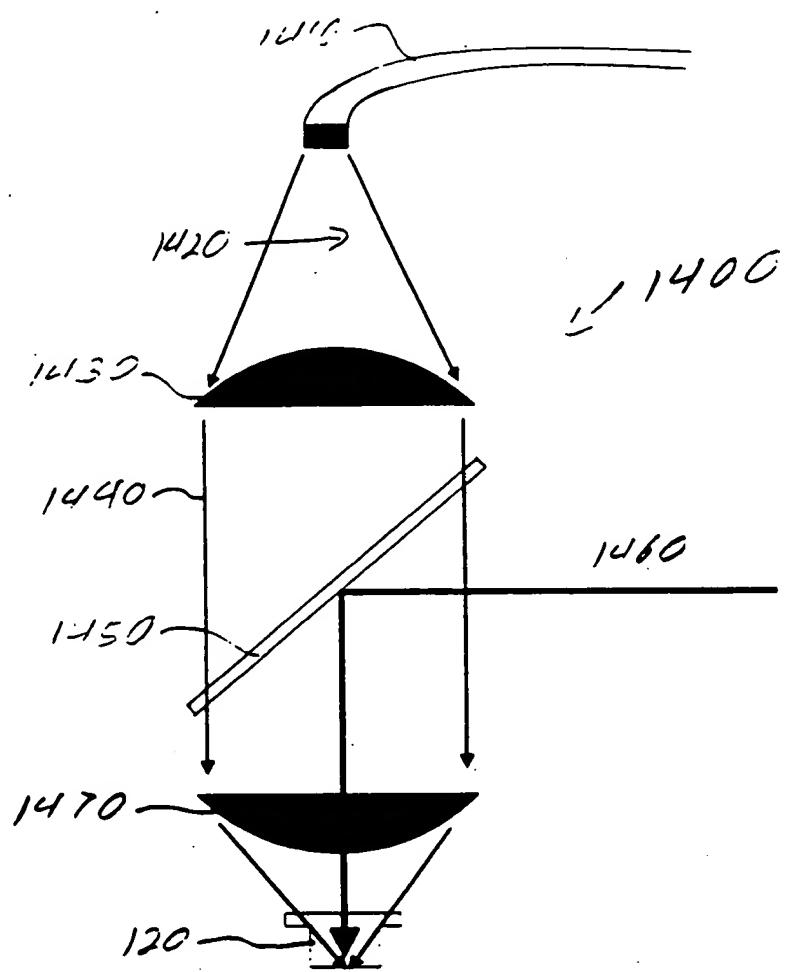


FIG 14

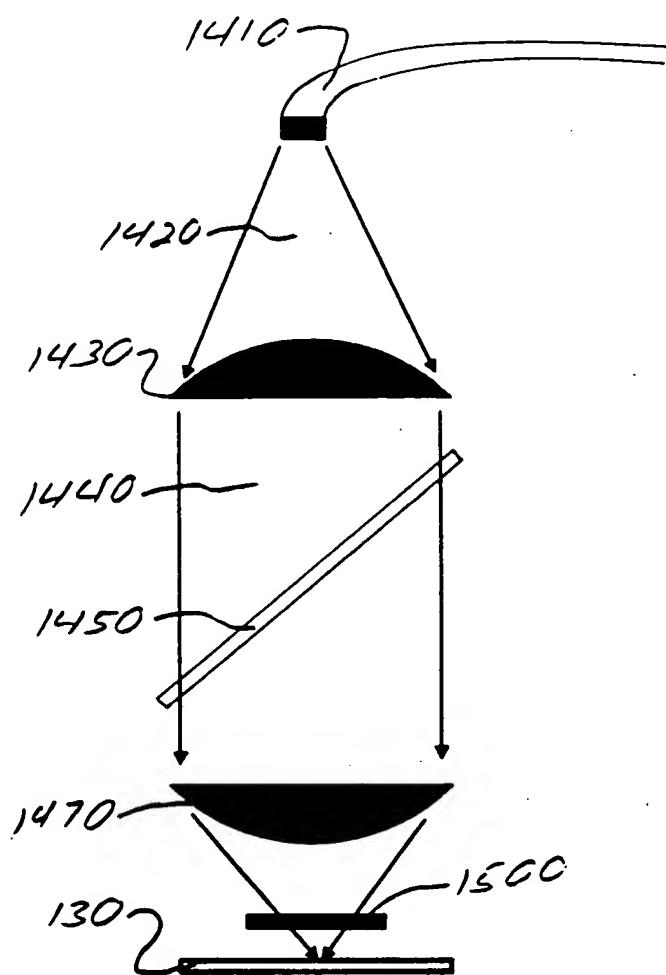


FIG 15

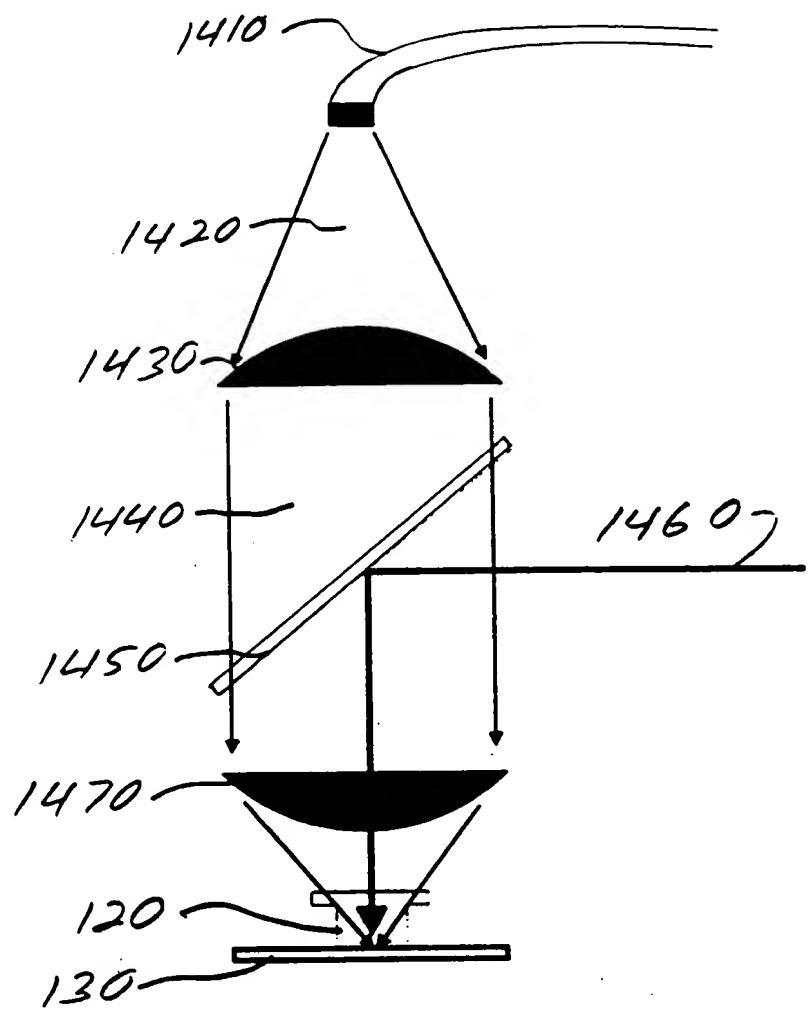


FIG. 16

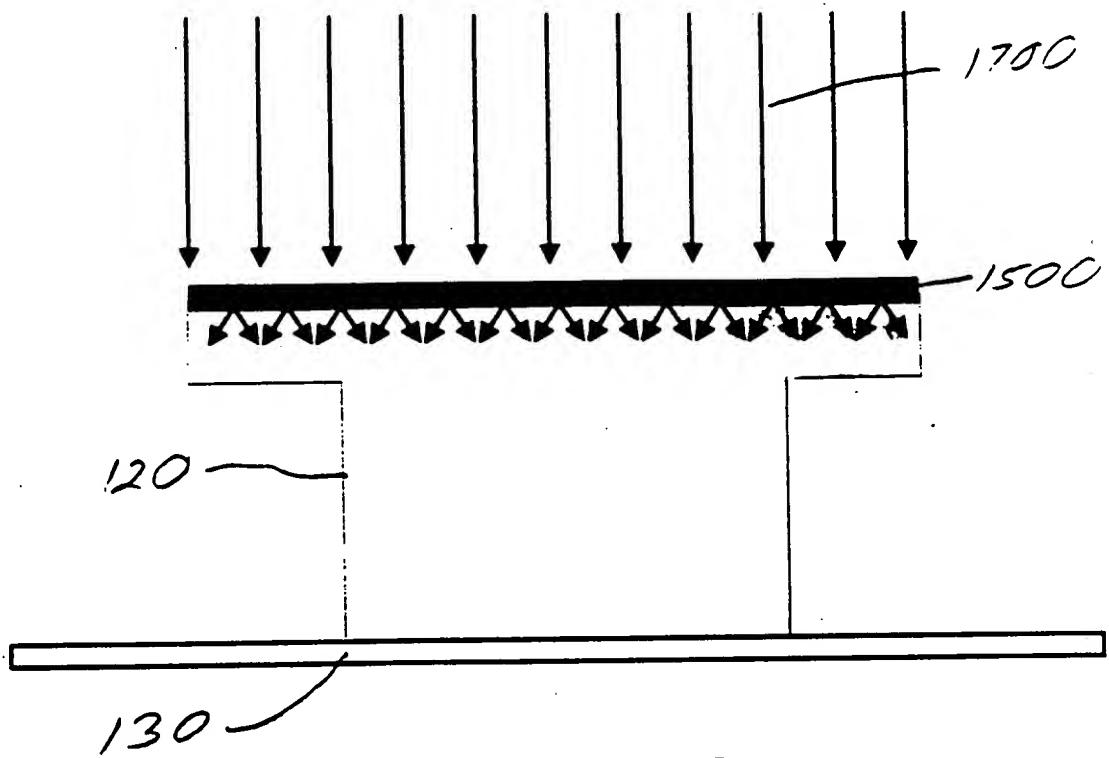


FIG. 17

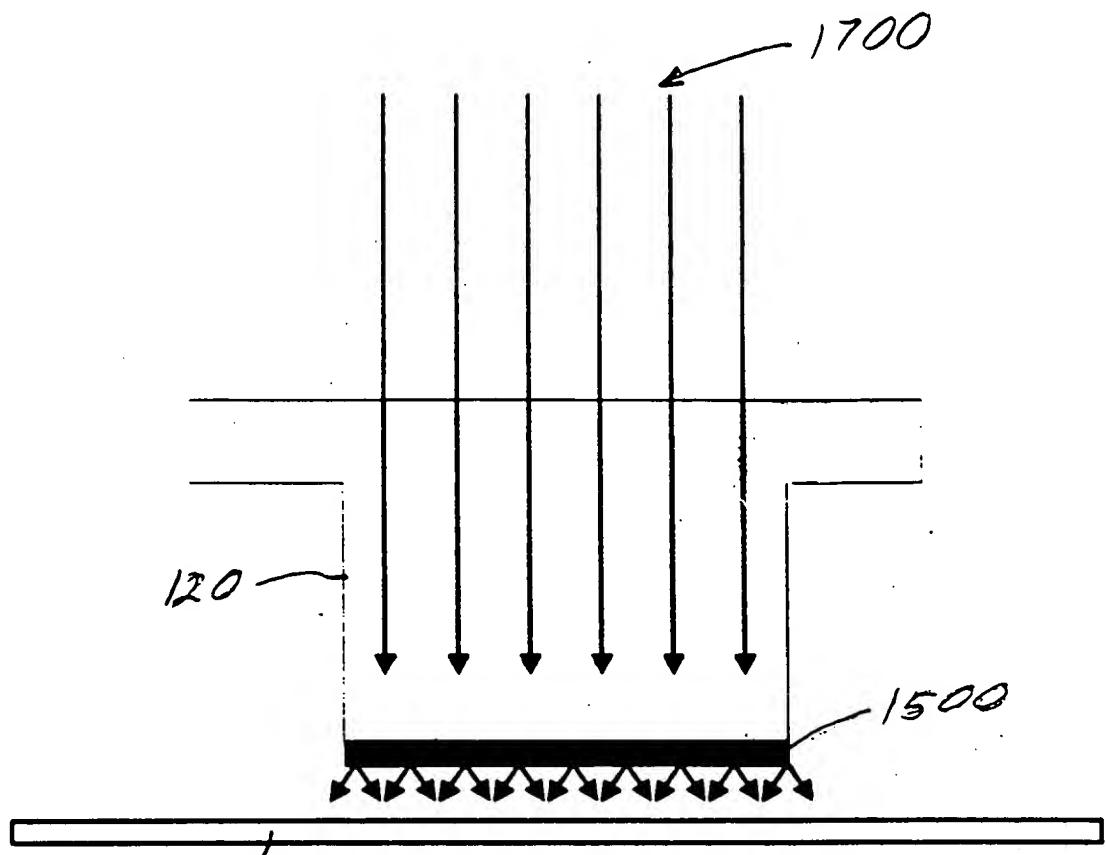


FIG. 18

09/121,691

PATENT
Attorney Docket No. 17726-704

**LASER CAPTURE MICRODISSECTION
VACUUM HOLD-DOWN**

Inventors: Thomas M. Baer; Mark A. Enright
David F. Head; and Christopher E. Todd

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is, under 35 U.S.C. § 120, a continuation of U.S. Ser. No. 09/018,452, filed February 4, 1998, now pending, which is a continuation-in-part of both U.S. Ser. No. 60/060,731, filed October 1, 1997, now pending, and U.S. Ser. No. 60/037,864, filed February 7, 1997, now abandoned, the entire contents of all which are hereby incorporated herein by reference as if fully set forth herein.

10

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to the field of laser capture microdissection (LCM). More particularly, the invention relates to inverted microscopes that include specialized apparatus for performing LCM. Specifically, a preferred implementation of the invention relates to an inverted microscope that includes a cap handling subsystem, an illumination/laser optical subsystem, a vacuum chuck subsystem, and a

manual joystick subsystem. The invention thus relates to inverted microscopes of the type that can be termed laser capture microdissection inverted microscopes.

5

Discussion of the Related Art

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from 10 the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

15 A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene 20 vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of 25 cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the

tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes
5 localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue
10 section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the
15 tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ
20 breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

25 Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research.

For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

The LCM technique is generally described in the recently published article: *Laser Capture Microdissection*, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

5 The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is
10 mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

15 While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

20 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for
laser capture microdissection. There is also a particular need for an
5 improved method of laser capture microdissection.

- A first aspect of the invention is implemented in an embodiment that
is based on a laser capture microdissection method, comprising: providing a
sample that is to undergo laser capture microdissection; positioning said
sample within an optical axis of a laser capture microdissection instrument;
10 providing a transfer film carrier having a substrate surface and a laser
capture microdissection transfer film coupled to said substrate surface;
placing said laser capture microdissection transfer film in juxtaposition with
said sample with a pressure sufficient to allow laser capture microdissection
transfer of a portion of said sample to said laser capture microdissection
15 transfer film, without forcing nonspecific transfer of a remainder of said
sample to said laser capture microdissection film; and then transferring a
portion of said sample to said laser capture microdissection transfer film,
without forcing nonspecific transfer of a remainder of said sample to said
laser capture microdissection transfer film.
- 20 A second aspect of the invention is implemented in an embodiment
that is based on a laser capture microdissection instrument, comprising: an
inverted microscope including: an illumination/laser optical subsystem; a
translation stage coupled to said illuminator/laser optical subsystem; a cap
handling subsystem coupled to said translation stage; a vacuum chuck
25 subsystem coupled to said translation stage; and a manual joystick
subsystem coupled to said translation stage.

These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

10

BRIEF DESCRIPTION OF THE DRAWINGS

A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

20
25 FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention;

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1;

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

5 FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention;

10 FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention;

FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6;

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention;

15 FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8;

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention;

20 FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10;

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention;

25 FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention;

FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention;

5 FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention;

FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention;

10 FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention; and

FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

15 **DESCRIPTION OF PREFERRED EMBODIMENTS**

The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed 20 in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997 entitled "Laser Capture Microdissection Device," (Docket No. ARCT-25 002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed

October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as

polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is 5 picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be 10 manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement. Further, this simultaneous movement can be effected with in a single 15 handed manner. The acquisition of samples is thus made quicker.

Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication 20 of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a function of operator hand movement.

In addition, the joystick provides tactile feedback not available with 25 electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X

and Y) as a function of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

5 Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185.
10 After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.
15

20 Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be
25 appreciated from FIG. 2B that the illumination beam path includes delivery

of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which can include a camera. In idle mode, the laser beam path 310 provides a visible low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second, preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength

of the laser is 810 nannometers. In a preferred embodiment the spot size of the laser at the EVA material located on microcentrifuge tube cap 120 is variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more preferably from 5 to 30 microns. These ranges are relatively preferred when
5 designing the optical subsystem. From the standpoint of the clinical operator, the widest spot size range is the most versatile. A lower end point in the spot size range on the order of 5 microns is useful for transferring single cells.

Suitable lasers can be selected from a wide power range. For
10 example, a 100 watt laser can be used. On the other hand, a 50 mW laser can be used. The laser can be connected to the rest of the optical subsystem with a fiber optical coupling. Smaller spot sizes are obtainable using diffraction limited laser diodes and/or single mode fiber optics. Single mode fiber allows a diffraction limited beam.

15 While the laser diode can be run in a standard mode such as TEM₀₀, other intensity profiles can be used for different types of applications. Further, the beam diameter could be changed with a stepped lens instead of lens 350.

20 Changing the beam diameter permits the size of the portion of the sample that is acquired to be adjusted. Given a tightly focused initial condition, the beam size can be increased by defocusing. Given a defocused initial condition, the beam size can be decreased by focusing. The change in focus can be in fixed amounts. The change in focus can be obtained by means of indents on a movable lens mounting and/or by means of optical
25 glass steps. In any event, increasing/decreasing the optical path length is the effect that is needed to alter the focus of the beam, thereby altering the spot

size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic), and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial. Fluorescence emitted by the sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for damping vertical motion of the cap handling mechanic subassembly 110.

5 The dampener 510 is adapted to lower the microcentrifuge tube cap 120 down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable of retarding the vertical motion of the subassembly 110 so as not to generate an impulse. As

10 the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before

15 the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to

20 microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By

25 raising the arm, the weight 530 is first picked off the microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 is picked up off of the

slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is

lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the
5 microcentrifuge tube cap 120 off the slide (not shown).

The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture
10 microdissection transfer film is approximately 0.26 square inches.

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

15 Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the
20 microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to
25 the position depicted in FIGS. 6-7).

Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and

the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

5 The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

10 There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place. Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

15 Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

20 It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

25 Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430 collimates the light from the fiber optic 1410. The collimator lens 1430 can

be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens). A collimated beam 1440 from the collimator lens 1430 then passes though a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460 is coaxial with the white light illumination. Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG 010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470 5 can have a numerical aperture of approximately 0.8. This can be characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

10 Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that contains the cells to be sampled.

15 More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type 20 eliminates the need for refractive index matching of the sample. Such a scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

25 The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk or opal glass which is

a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example,
5 frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser. In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light
10 sufficiently.

Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed
15 into the microcentrifuge tube cap 120 so as to acquire the desired sample.

The purpose of the illuminator design is to provide a very high numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization,
20 this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important
25 because the sample is adjacent the bottom of the sample carrier and the

objective cannot move closer to the sample than the top of the sample carrier.

The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in visualization. This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

10 The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

15 In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

20 Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

25 The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a

speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is

pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the velocity should be such that the sample is thixotropically sheared.

5

Practical Applications of the Invention

A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases.
10 This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient
15 samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually
20 innumerable uses for the invention, all of which need not be detailed here.

Advantages of the Invention

A laser capture microdissection instrument and/or method
25 representing an embodiment of the invention can be cost effective and advantageous for at least the following reasons. The invention will replace

current methods with better technology that allows for more accurate and reproducible results. The invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent was specifically and individually indicated to be incorporated in its entirety by reference.

All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the

LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

5

10

It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
 - providing a sample that is to undergo laser capture microdissection;
 - positioning said sample on a translation stage of a laser capture microdissection instrument and within an optical axis of said laser capture microdissection instrument, said translation stage including a vacuum chuck having a beam path hole through which said optical axis extends;
 - holding a sample holder in a position over said beam path hole, said sample being located upon said sample holder;
 - providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface;
 - placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and
 - then
 - transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.
2. The method of claim 1, further comprising translating said sample holder with regard to said translation stage.

3. The method of claim 1, wherein holding a sample holder in said position over said beam path hole includes holding said sample holder with a force and modulating said force.
4. The method of claim 1, further comprising pulling a vacuum on said sample holder.
5. The method of claim 1, further comprising applying a force to an edge of said sample holder to move said sample holder with regard to said translation stage.
6. The method of claim 1, further comprising moving said sample holder in any direction parallel with a top surface of said translation stage without constraint.
7. A laser capture microdissection instrument, comprising:
 - a translation stage; and
 - a vacuum chuck coupled to said translation stage.
8. The laser capture microdissection instrument of claim 7, wherein said vacuum chuck includes a beam path hole.
9. The laser capture microdissection instrument of claim 8, wherein a top surface of said vacuum chuck includes a first manifold hole and a second manifold hole.

10. The laser capture microdissection instrument of claim 9, wherein a sample holder is placed over said beam path hole, said first manifold hole, and said second manifold hole.
11. The laser capture microdissection instrument of claim 10, wherein there is leakage around a perimeter of said sample holder which modulates a force holding said sample holder in place.
12. The laser capture microdissection instrument of claim 7, wherein said vacuum chuck includes a conduit.
13. The laser capture microdissection instrument of claim 12, wherein said conduit is connected to a circular manifold that is coupled to a first manifold hole and a second manifold hole.
14. The laser capture microdissection instrument of claim 7, wherein there are no structures that project above a top surface of said vacuum chuck.
15. The laser capture microdissection instrument of claim 7, further comprising a transfer film carrier handling subsystem connected to said translation stage.
16. The laser capture microdissection instrument of claim 7, further comprising an illumination/laser optical subsystem coupled to said translation stage.

17. The laser capture microdissection instrument of claim 7, further comprising a manual joystick subsystem connected to said translation stage.
18. An inverted microscope, comprising:
a translation stage; and
a vacuum chuck connected to said translation stage.
19. The laser capture microdissection instrument of claim 18, wherein said vacuum chuck includes a beam path hole.
20. The laser capture microdissection instrument of claim 19, wherein a top surface of said vacuum chuck includes a first manifold hole and a second manifold hole.
21. The laser capture microdissection instrument of claim 20, wherein a sample holder is placed over said beam path hole, said first manifold hole, and said second manifold hole.
22. The laser capture microdissection instrument of claim 21, wherein there is leakage around a perimeter of said sample holder which modulates a force holding said sample holder in place.
23. The laser capture microdissection instrument of claim 18, wherein said vacuum chuck includes a conduit.

24. The laser capture microdissection instrument of claim 23, wherein said conduit is connected to a circular manifold that is coupled to a first manifold hole and a second manifold hole.
25. The laser capture microdissection instrument of claim 18, wherein there are no structures that project above a top surface of said vacuum chuck.
26. The inverted microscope of claim 18, further comprising a transfer film carrier handling subsystem connected to said translation stage.
27. The inverted microscope of claim 18, further comprising an illumination/laser optical subsystem coupled to said translation stage.
28. The inverted microscope of claim 18, further comprising a manual joystick subsystem connected to said translation stage.

ABSTRACT OF THE DISCLOSURE

Systems and methods for laser capture microdissection are disclosed.

An inverted microscope includes a vacuum chuck subsystem that is adapted to hold a microscope slide down during the process of laser capture microdissection. The systems and methods provide the advantages of increased speed and much lower rates of contamination.

PUT IN
17726-
704

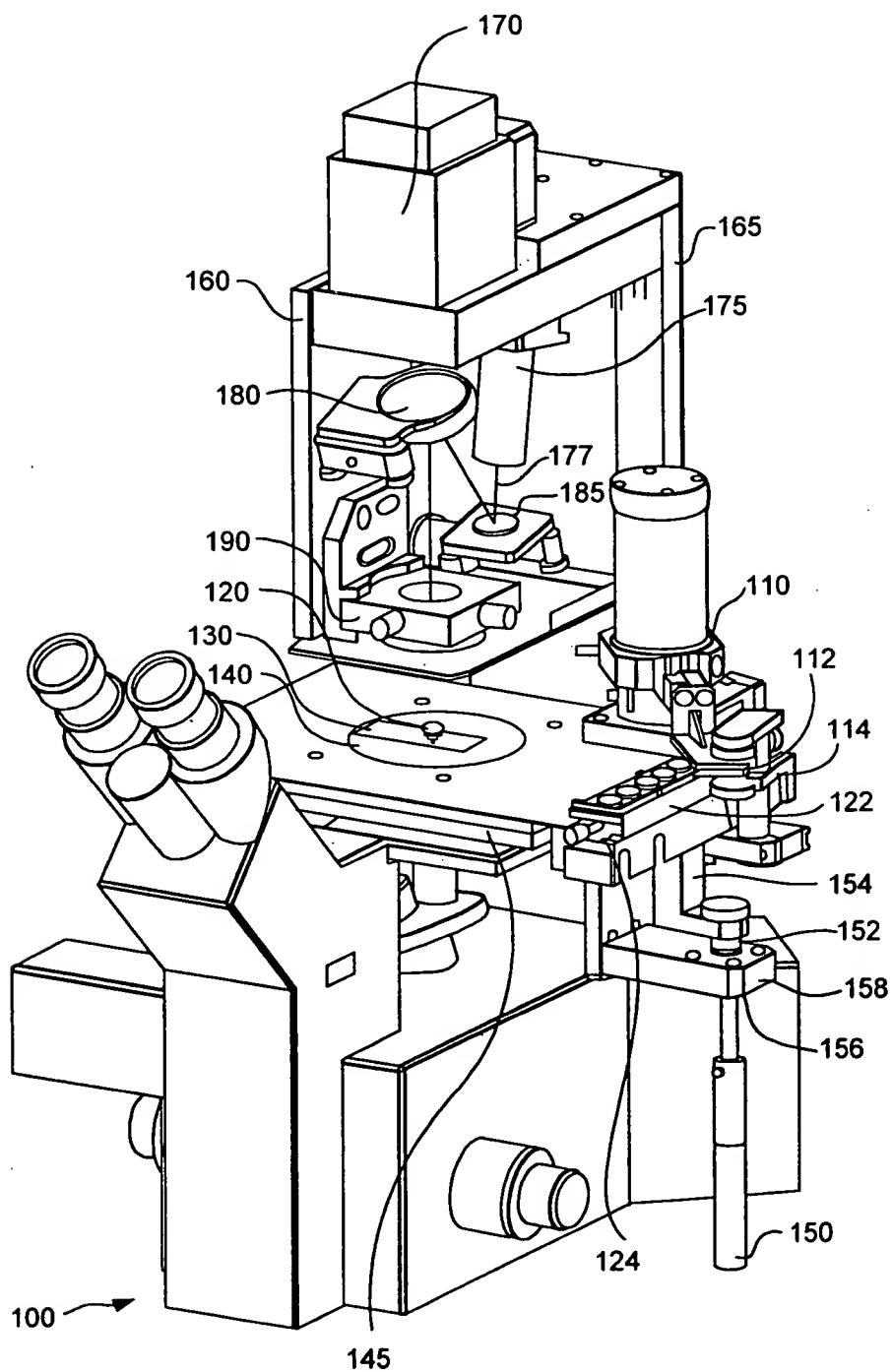


FIG. 1

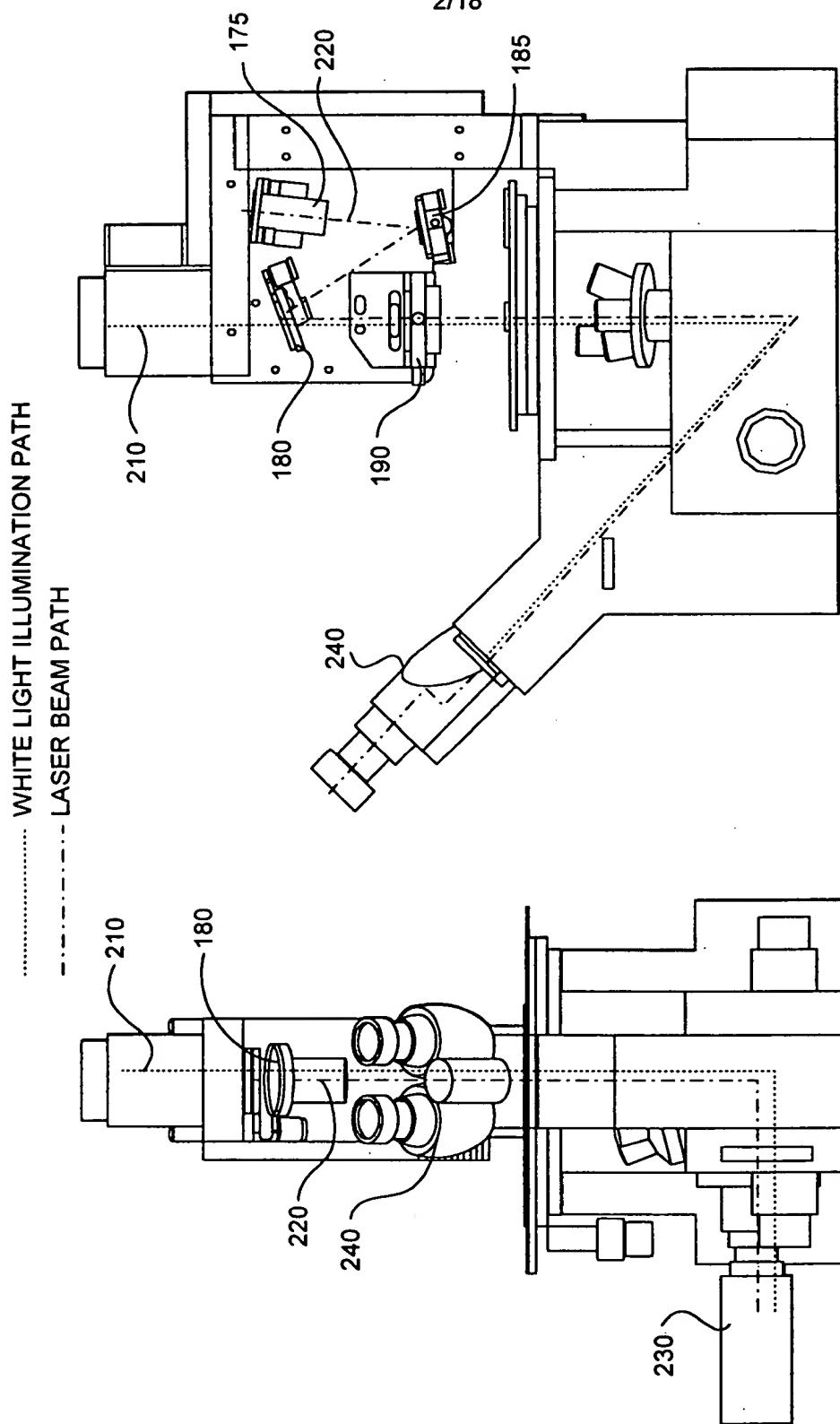


FIG. 2B

FIG. 2A

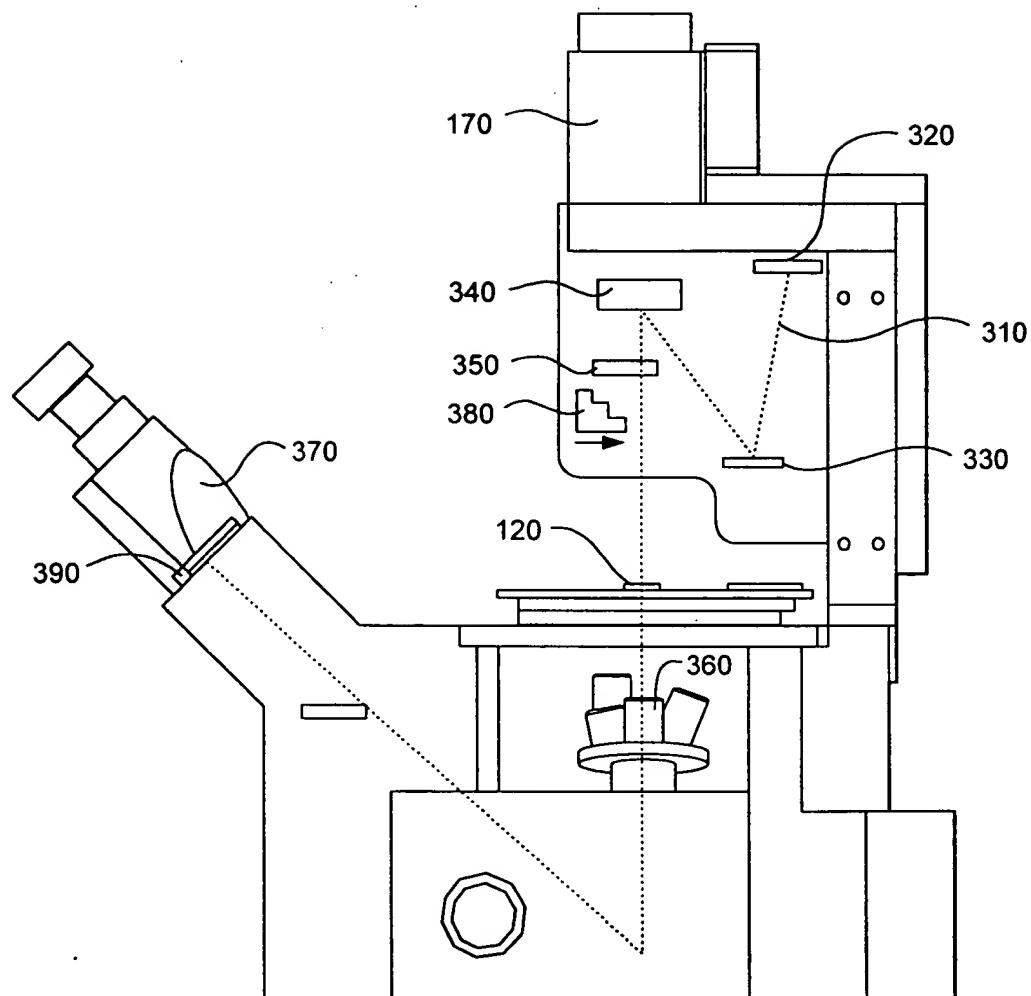


FIG. 3

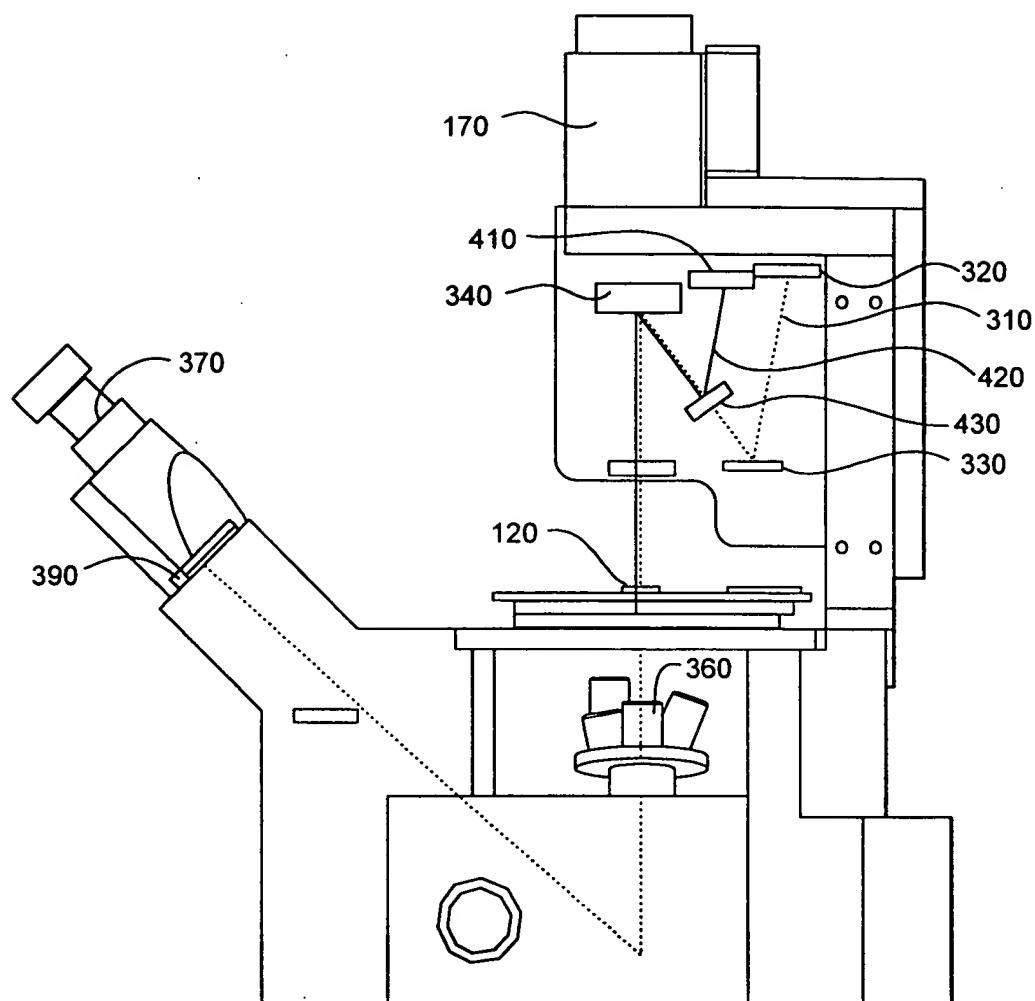


FIG. 4

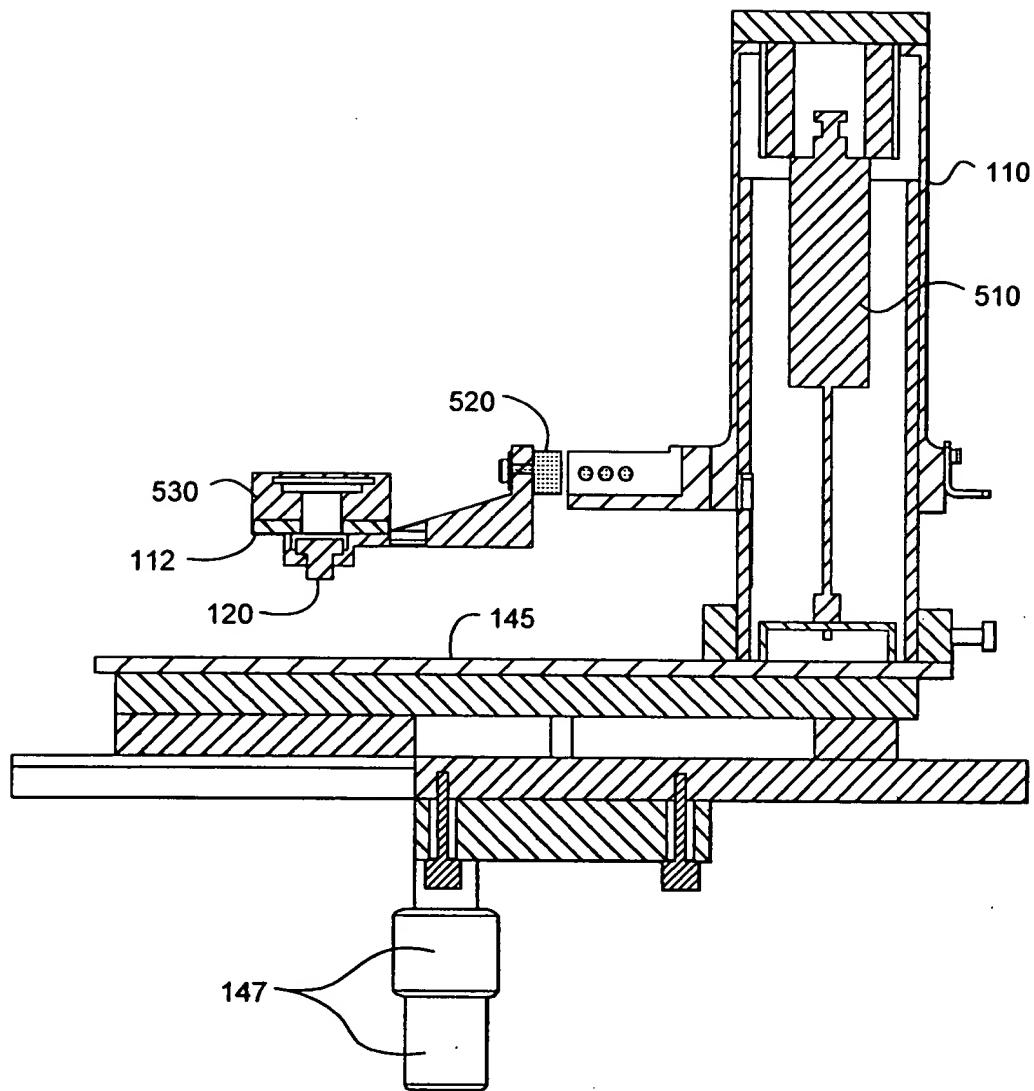


FIG. 5

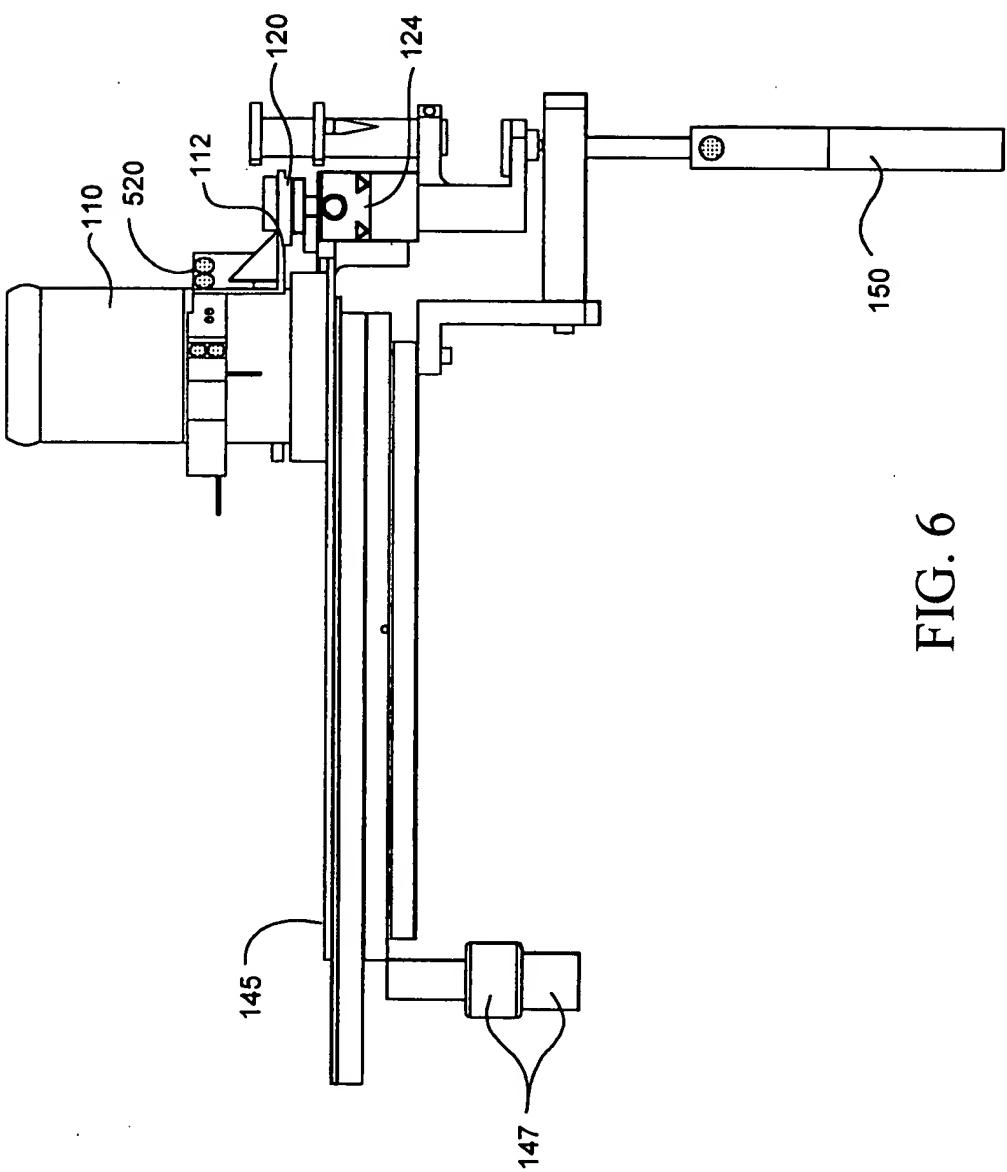


FIG. 6

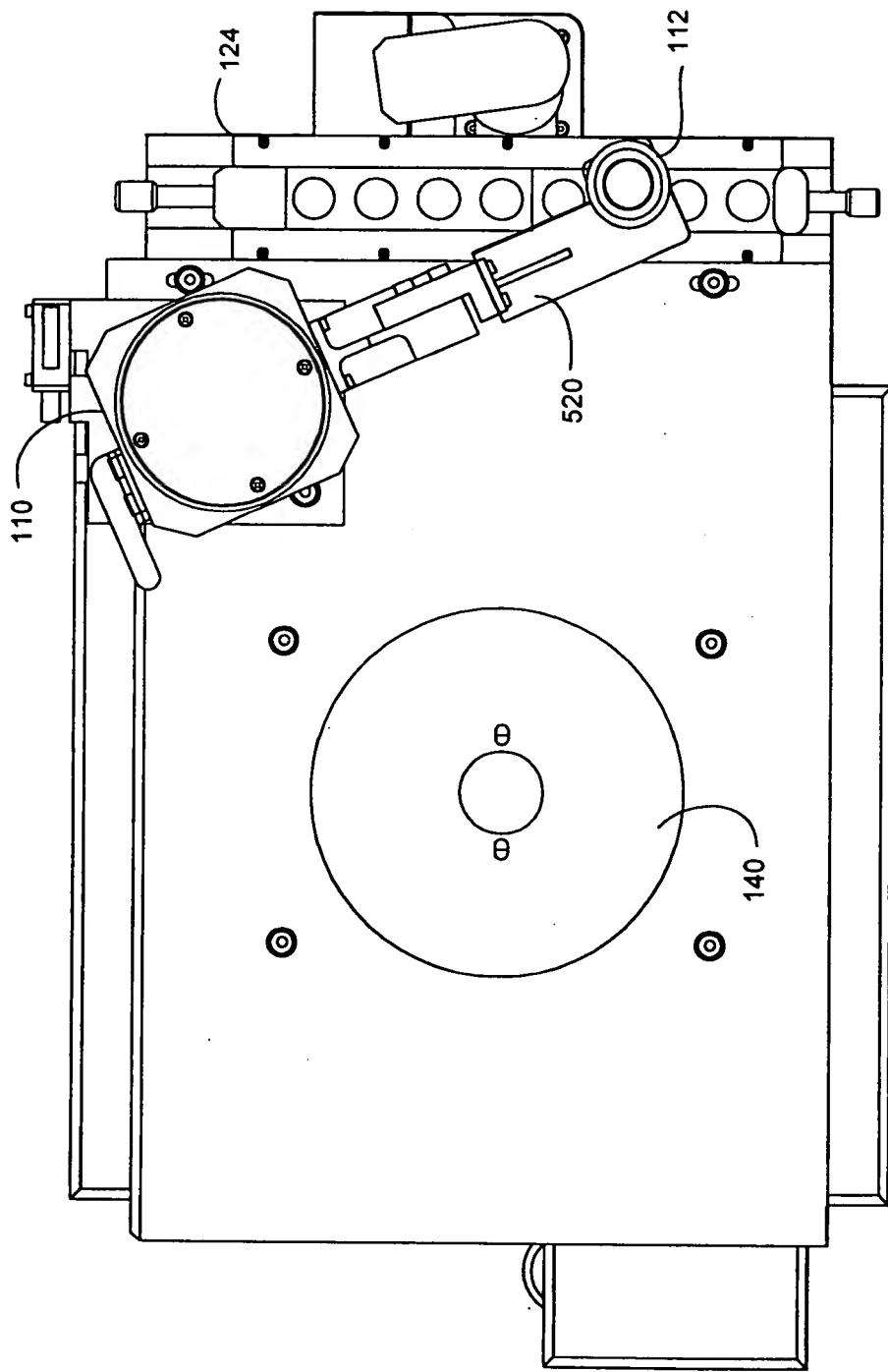


FIG. 7

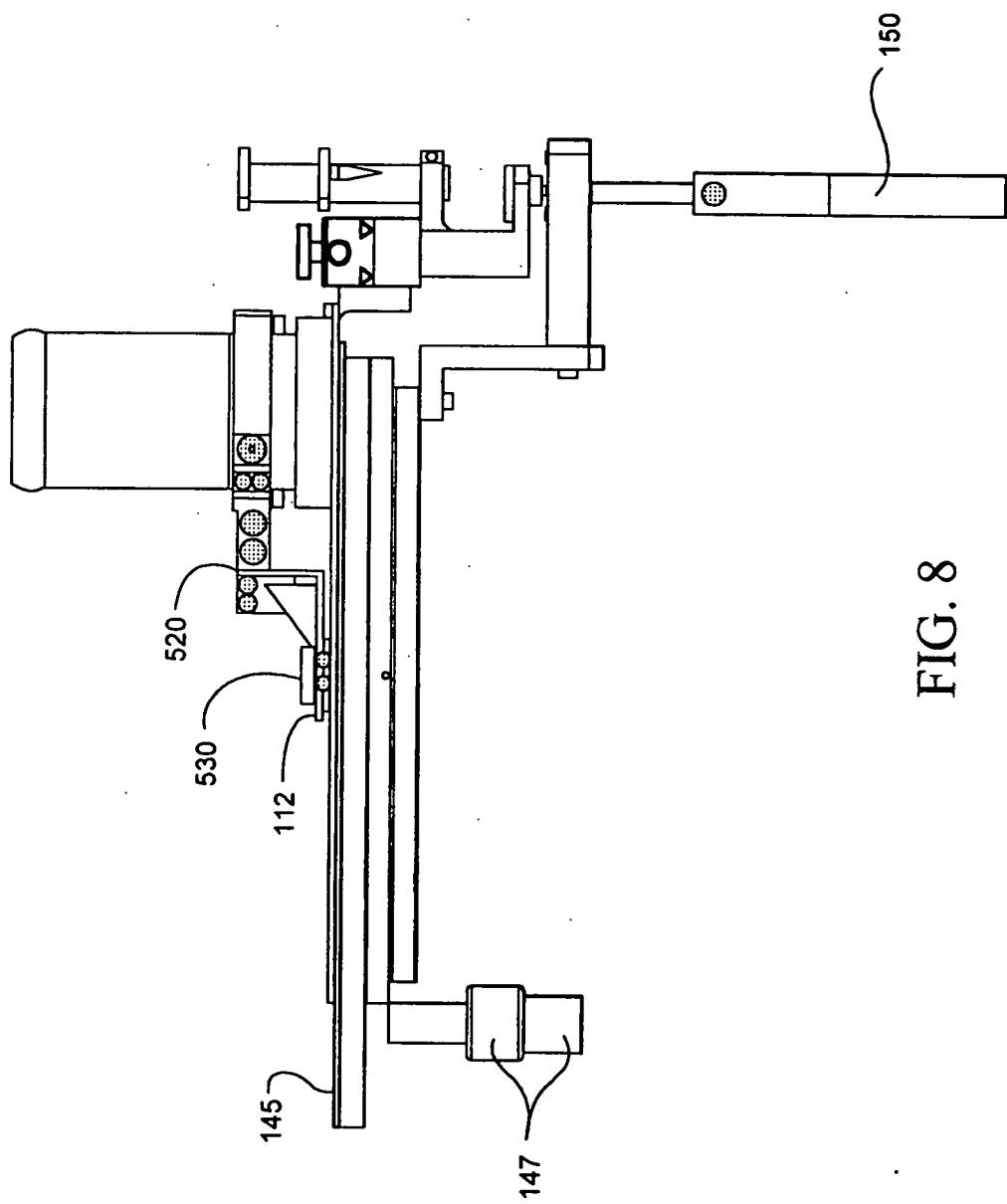


FIG. 8

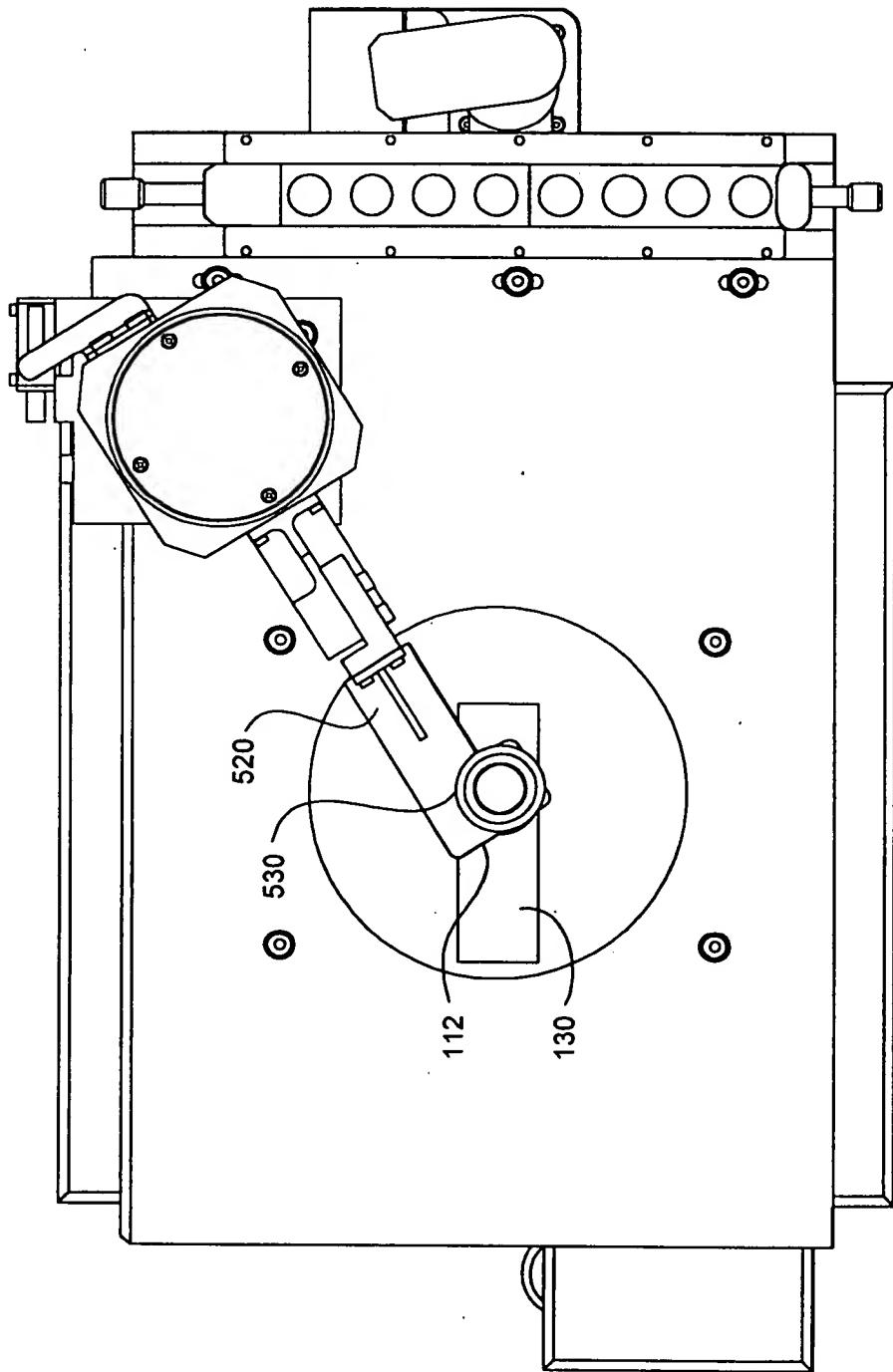


FIG. 9

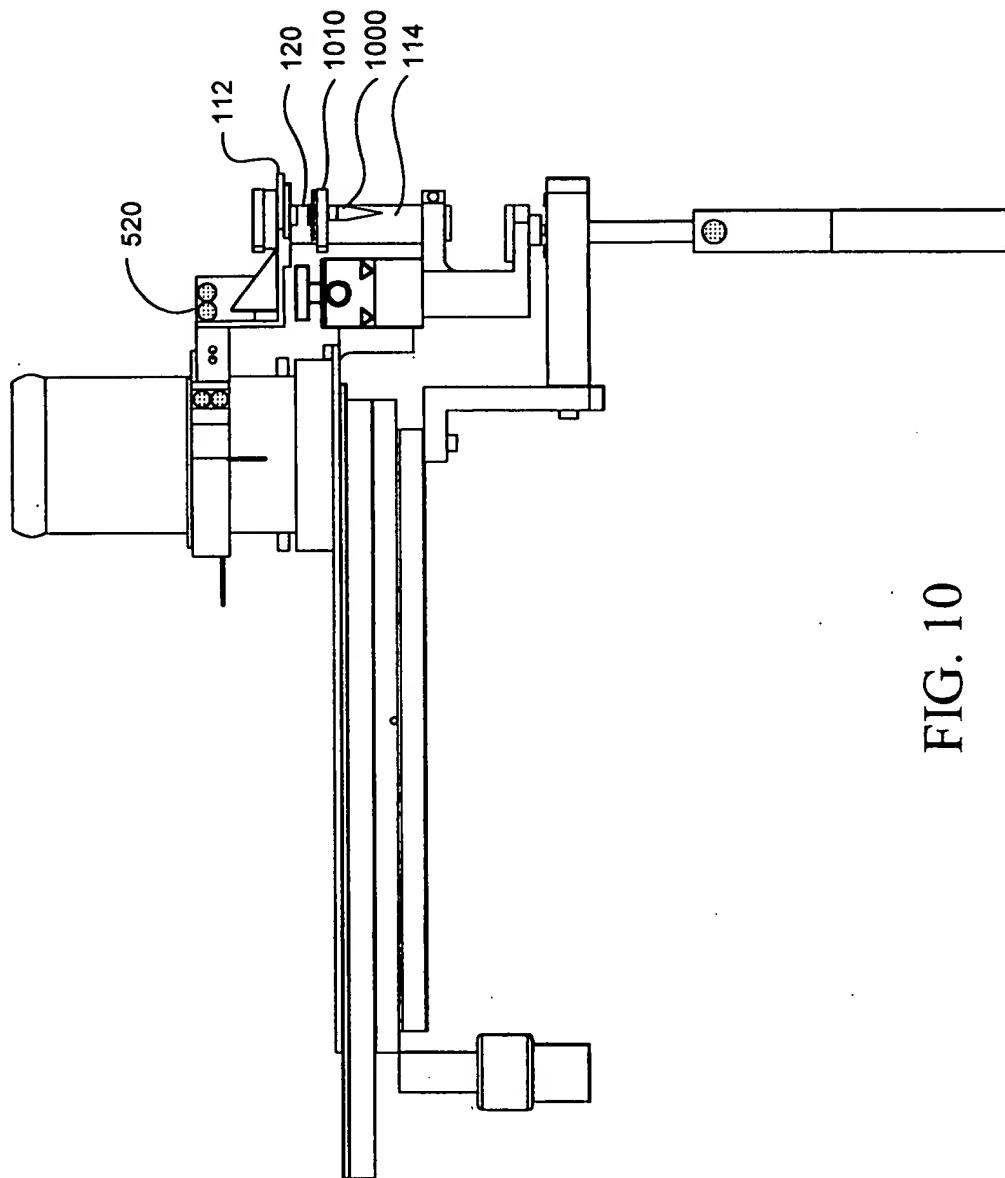


FIG. 10

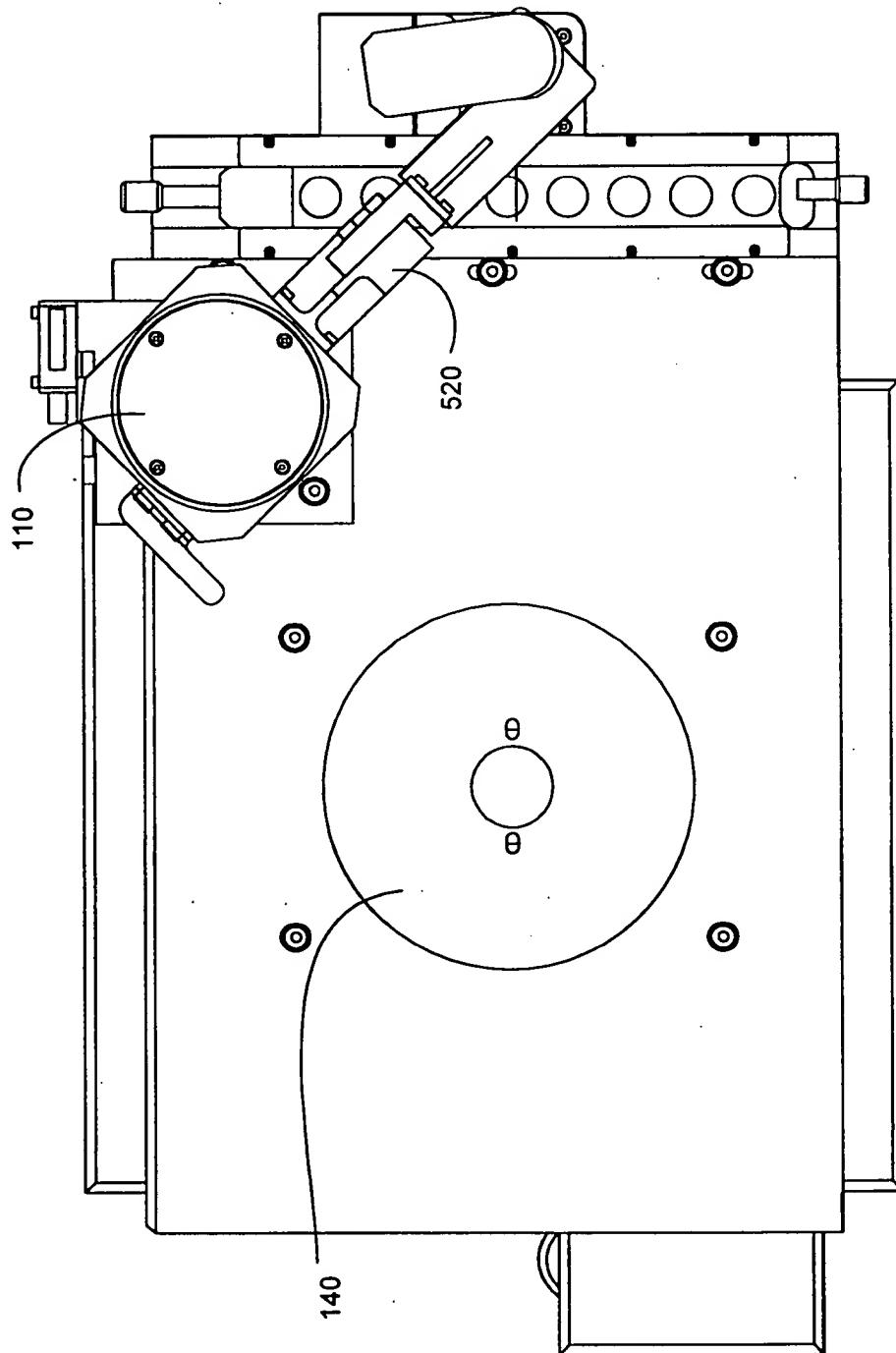


FIG. 11

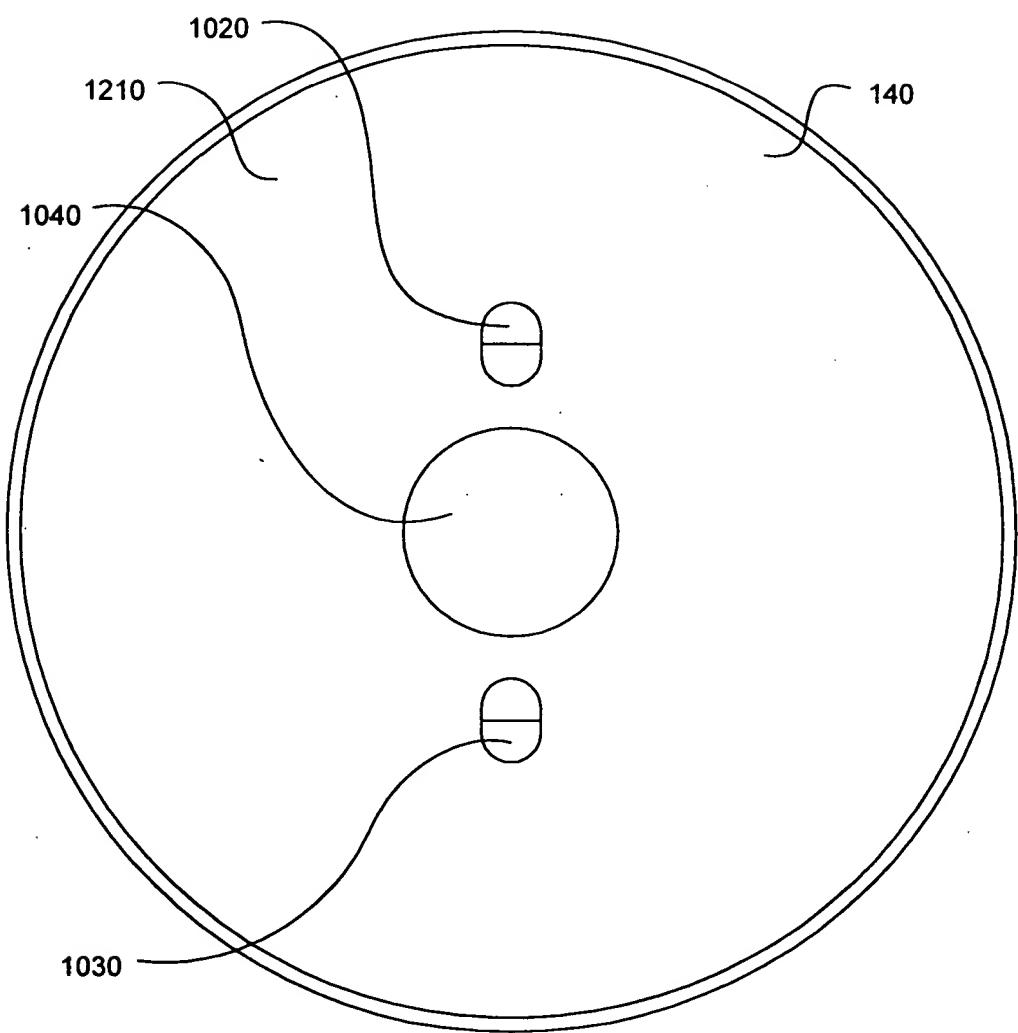


FIG. 12

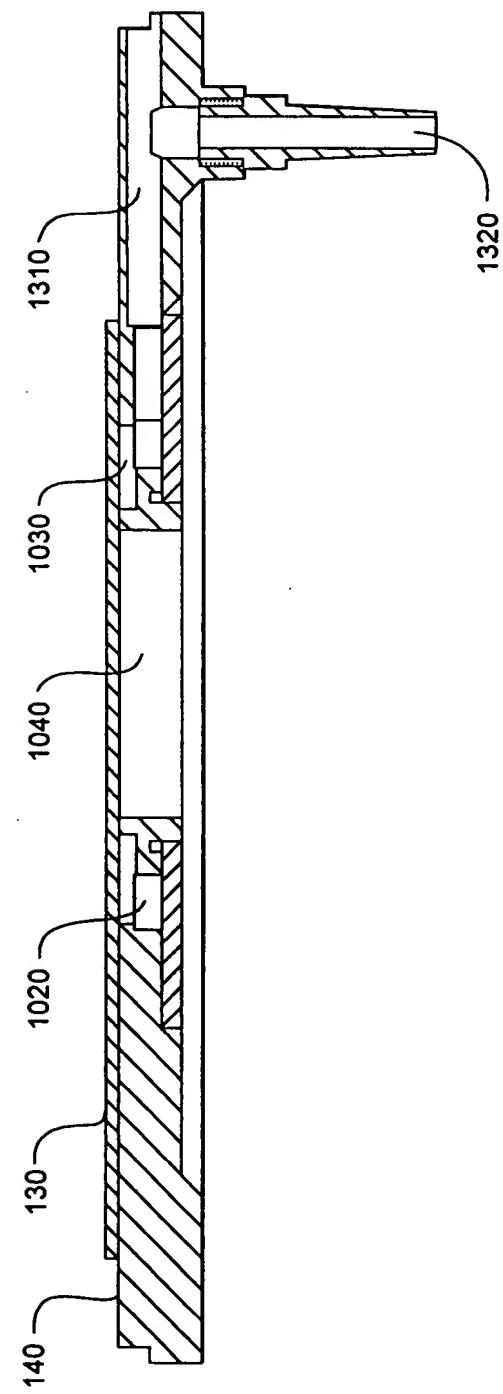


FIG. 13

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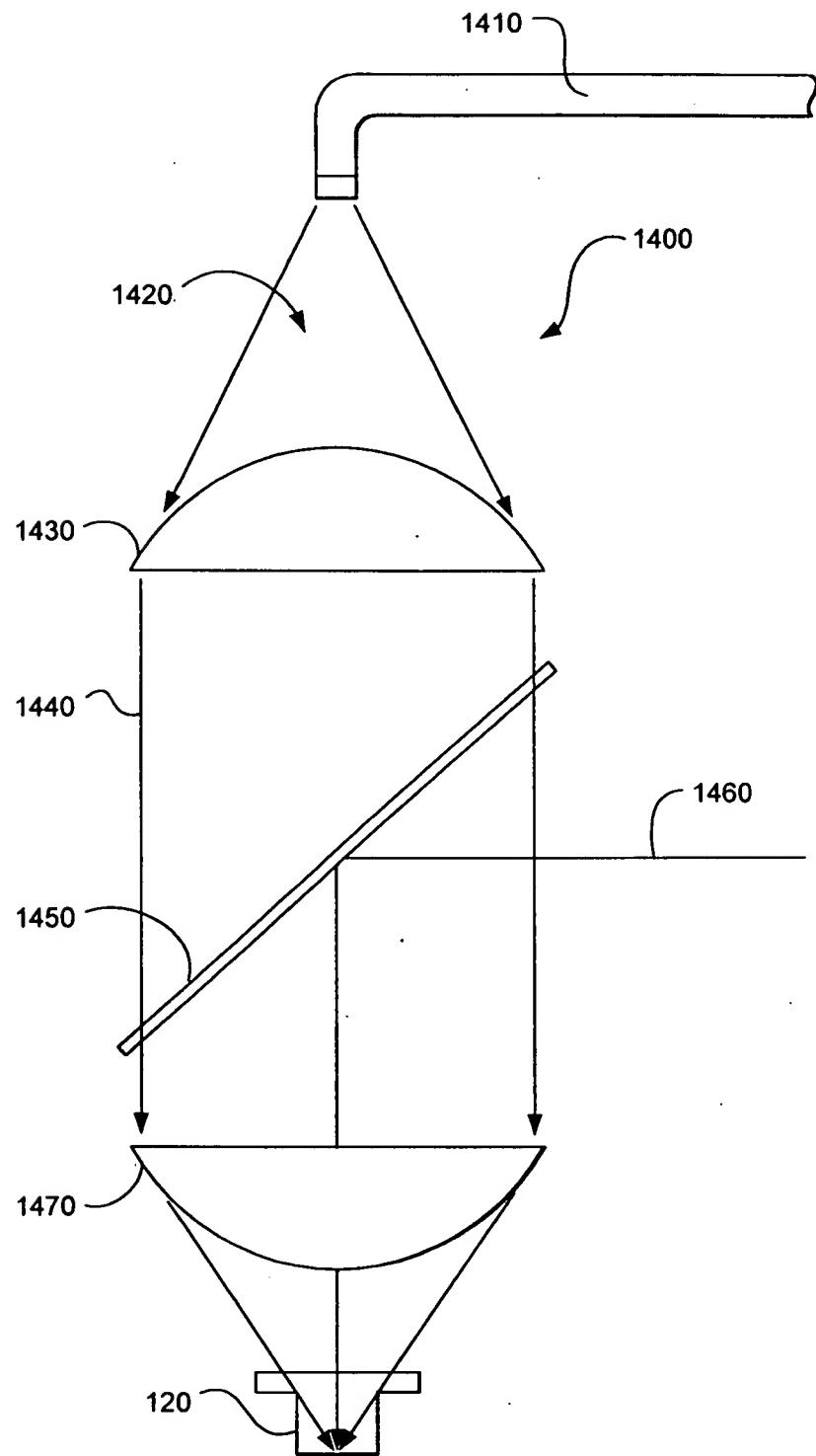


FIG. 14

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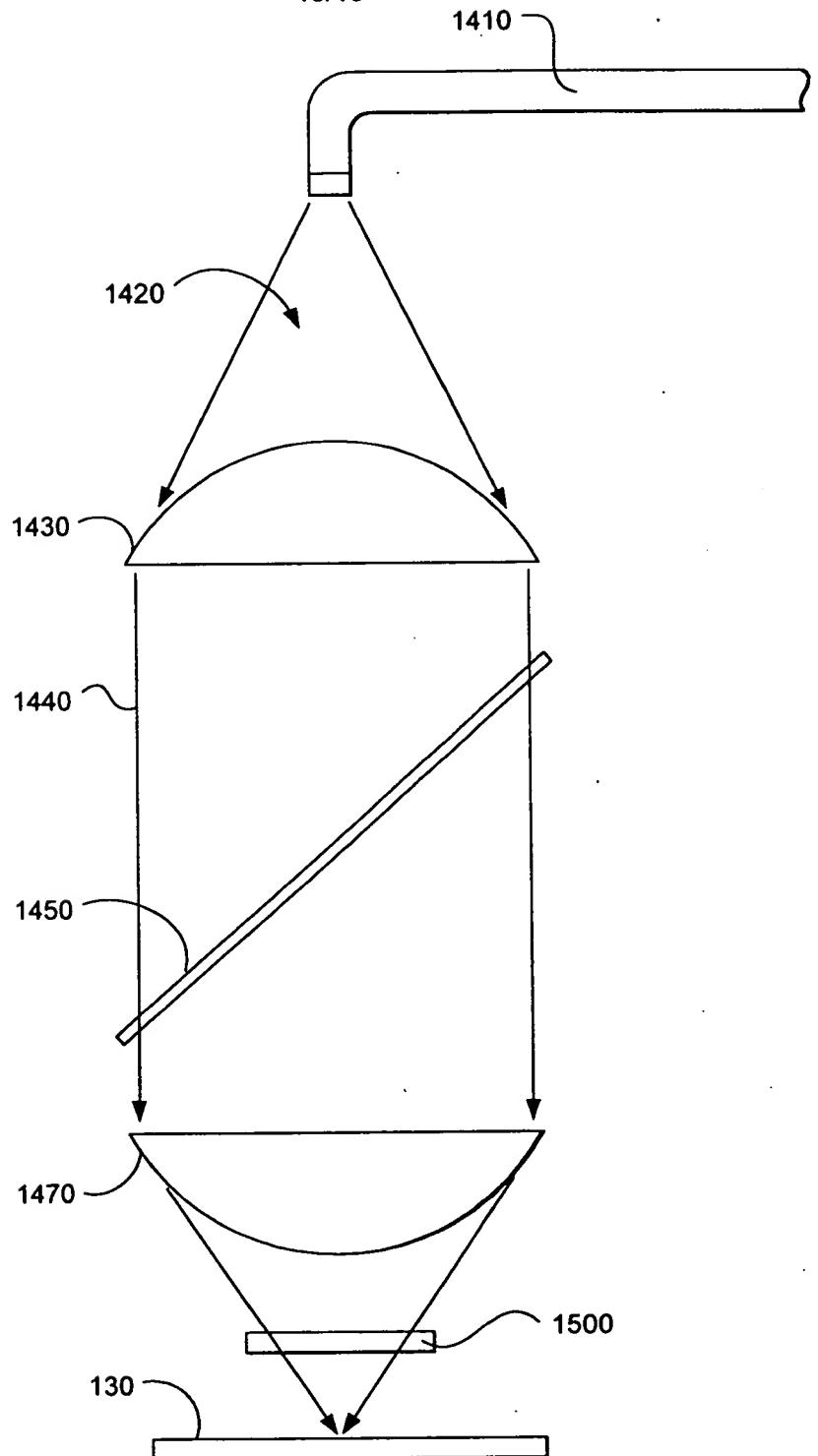


FIG. 15

16/18

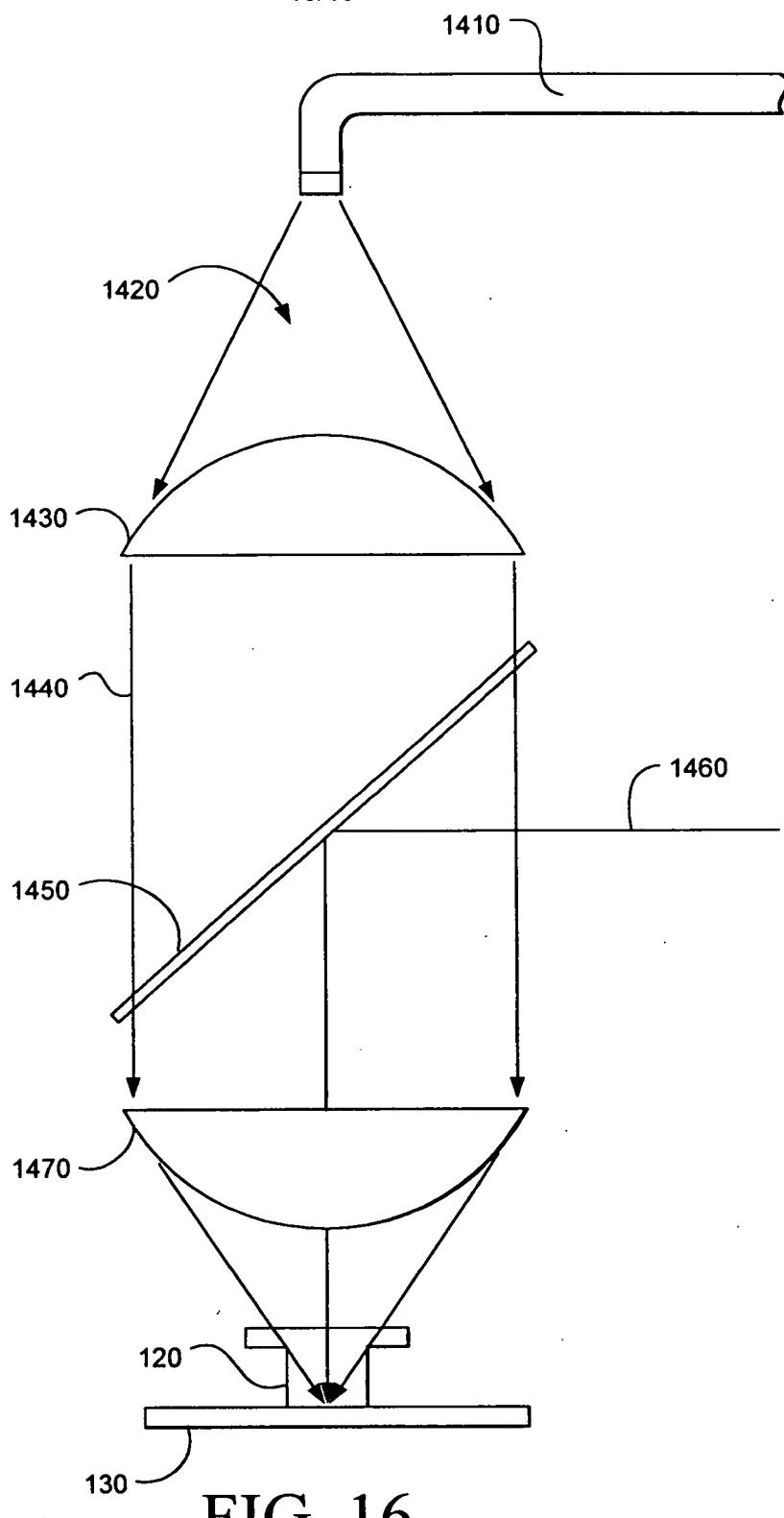


FIG. 16

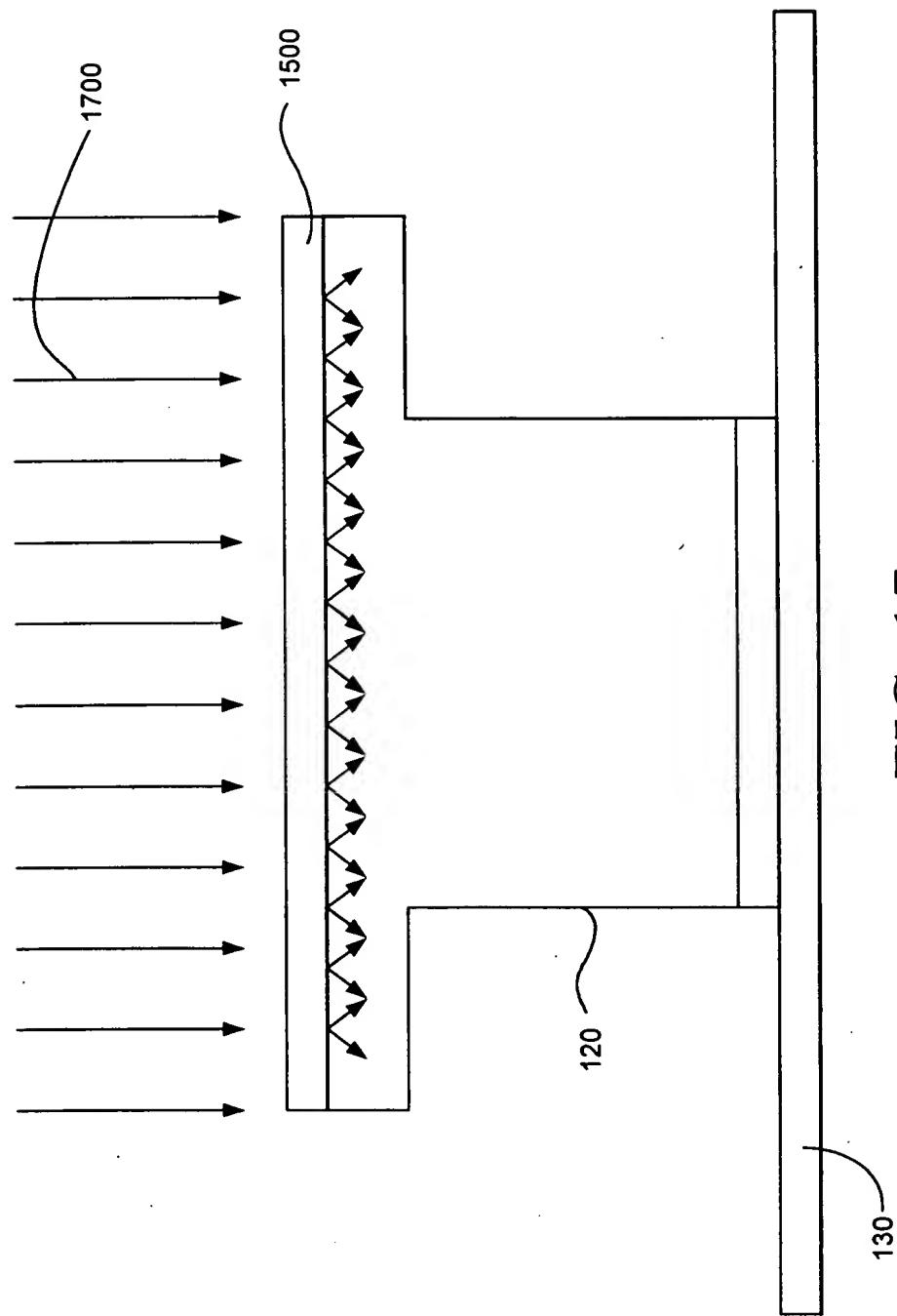


FIG. 17

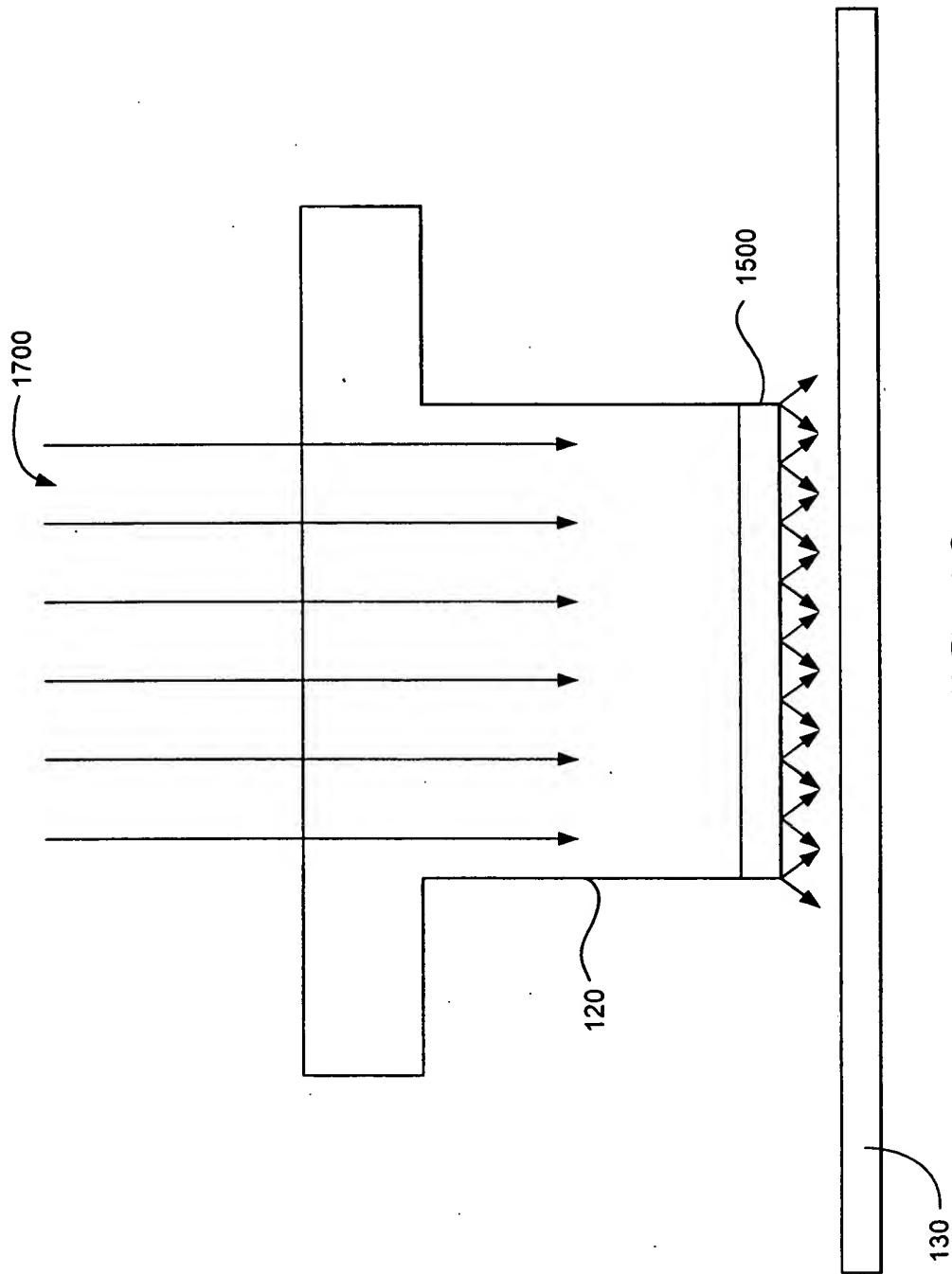


FIG. 18

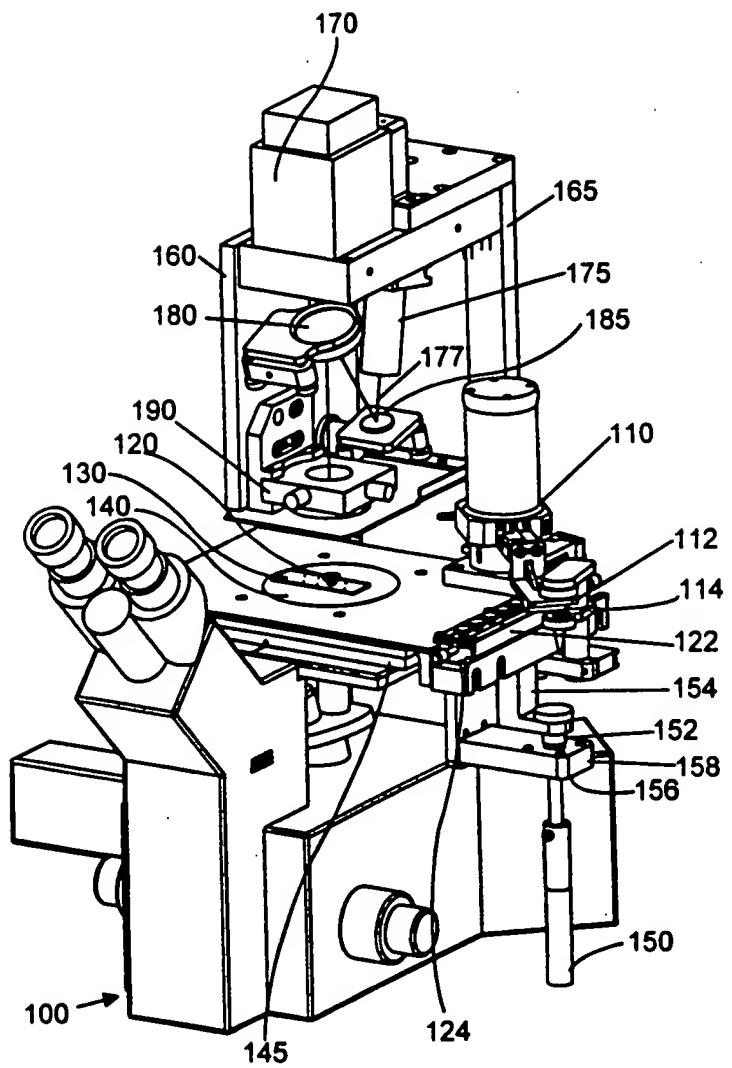
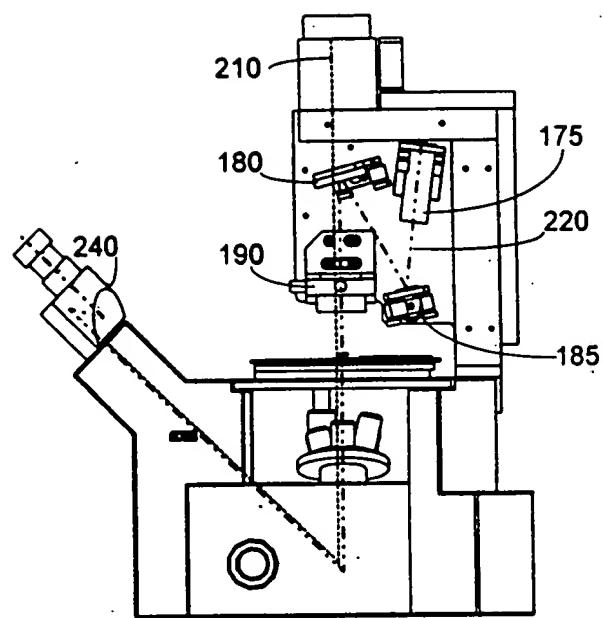
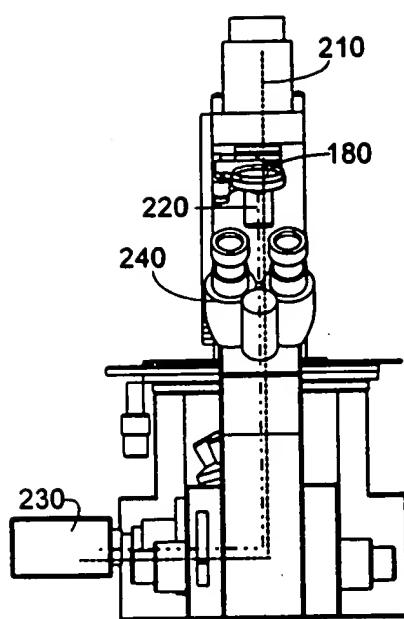


FIG. 1



— WHITE LIGHT ILLUMINATION PATH
— LASER BEAM PATH

FIG. 2A

FIG. 2B

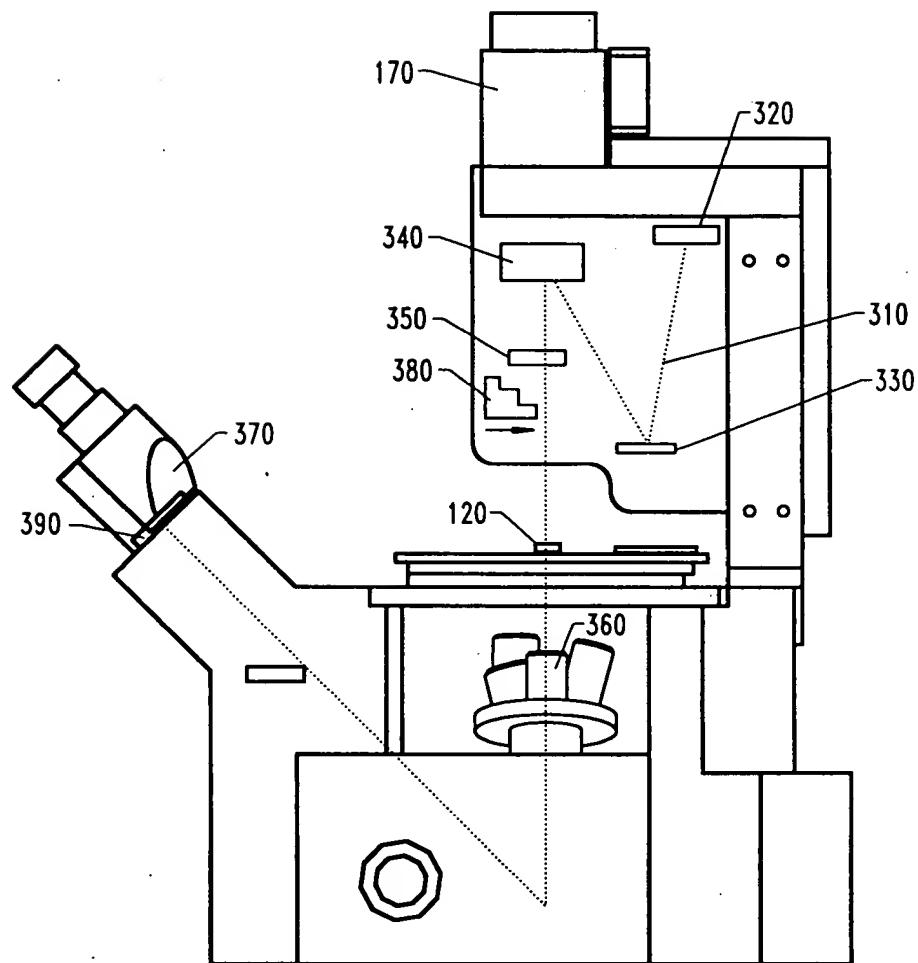


FIG. 3

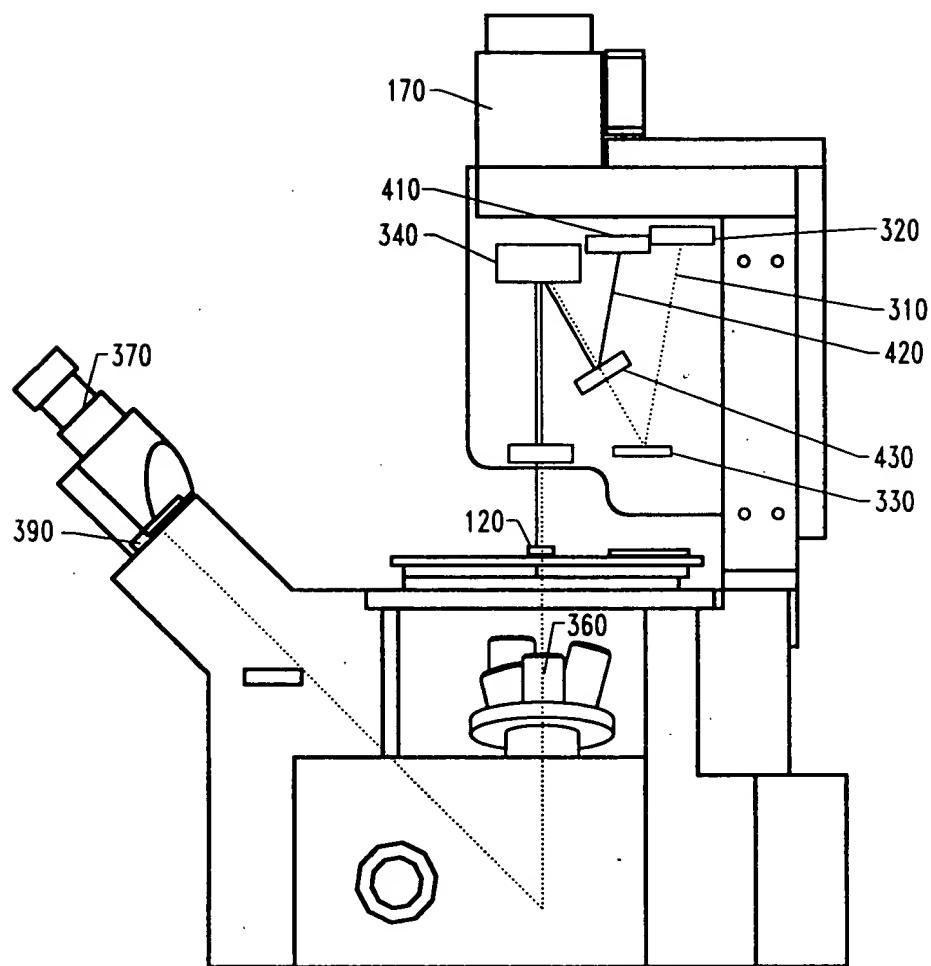


FIG. 4

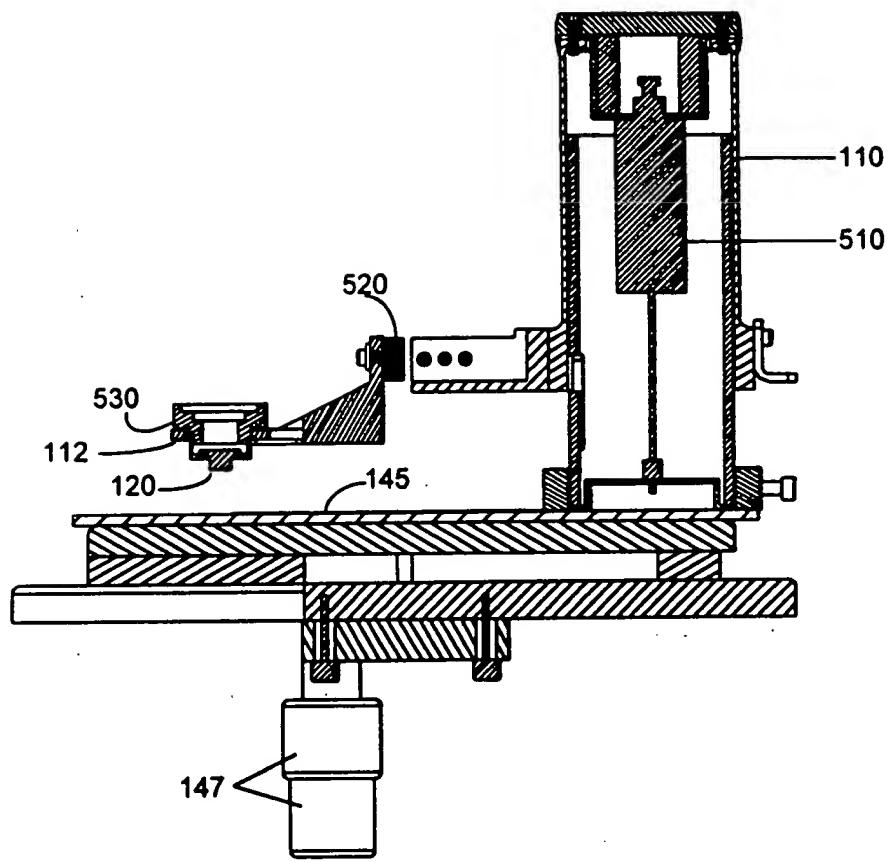


FIG. 5

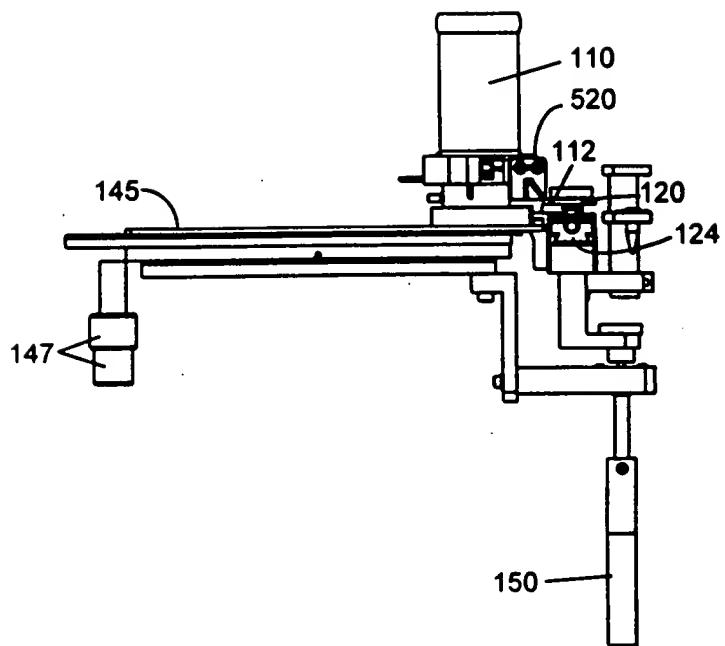


FIG. 6

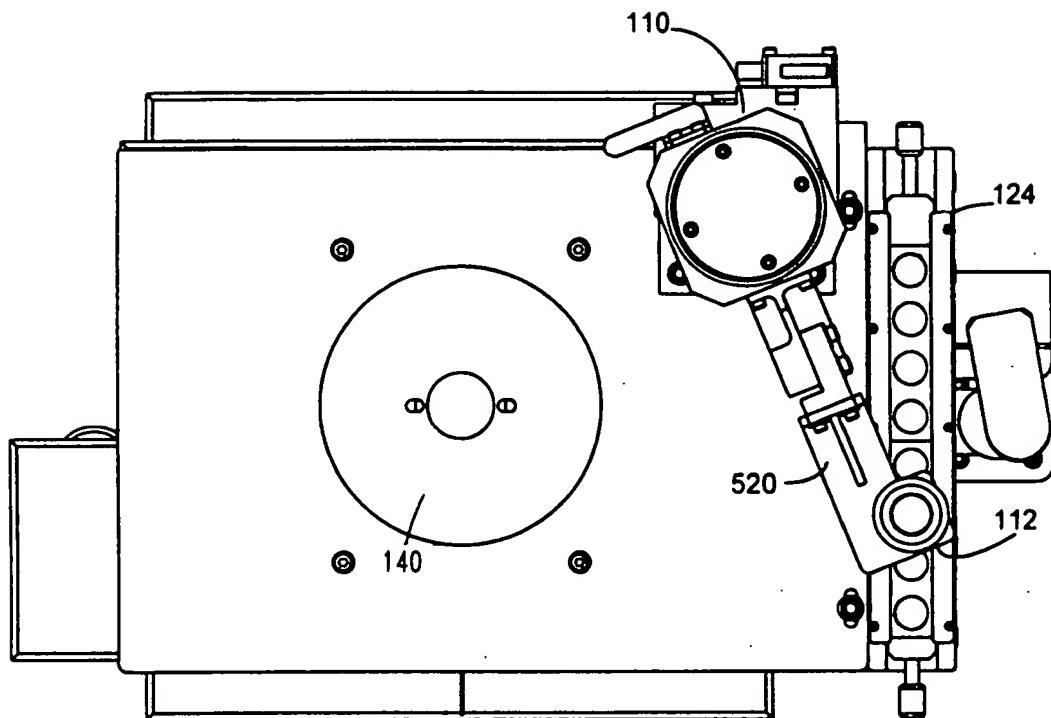


FIG. 7

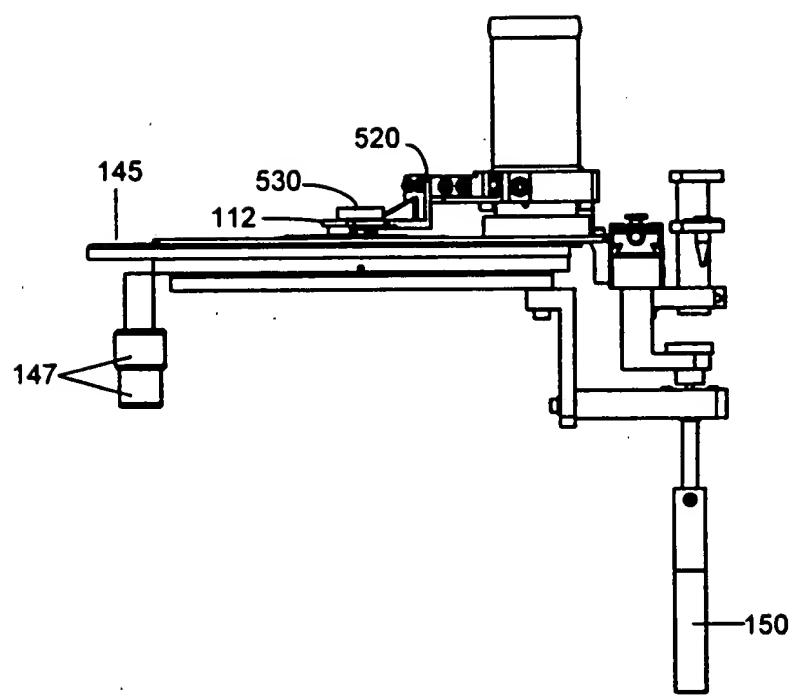


FIG. 8

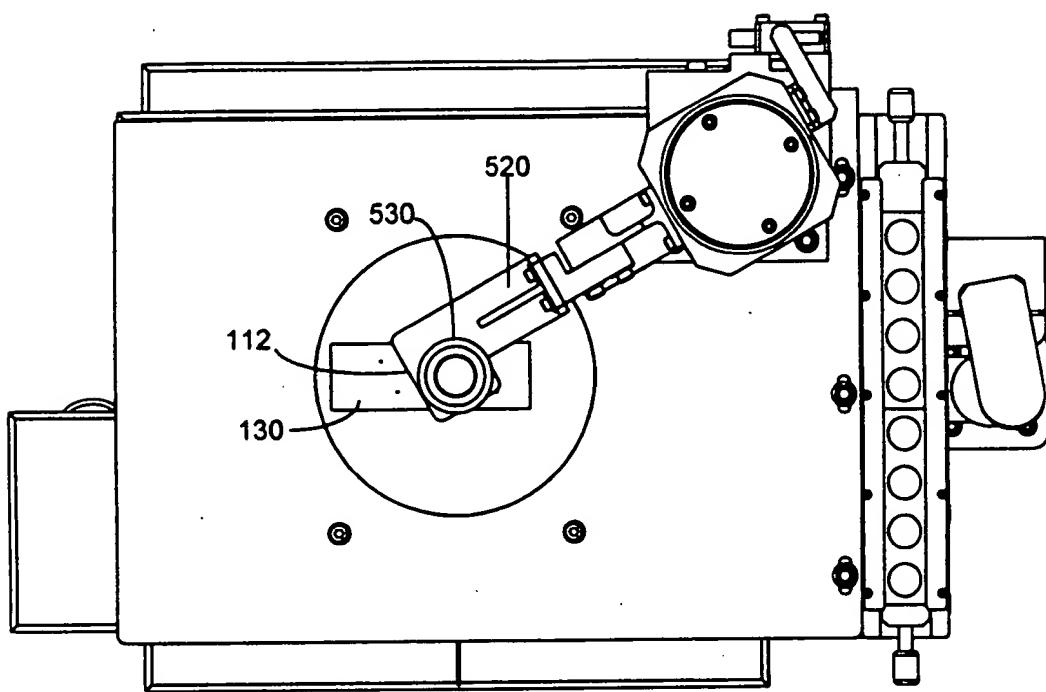


FIG. 9

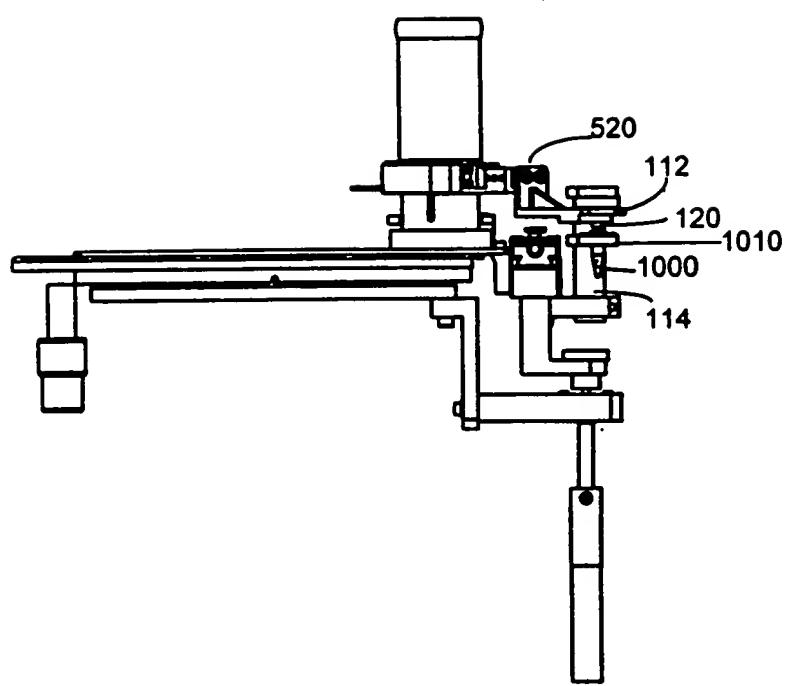


FIG. 10

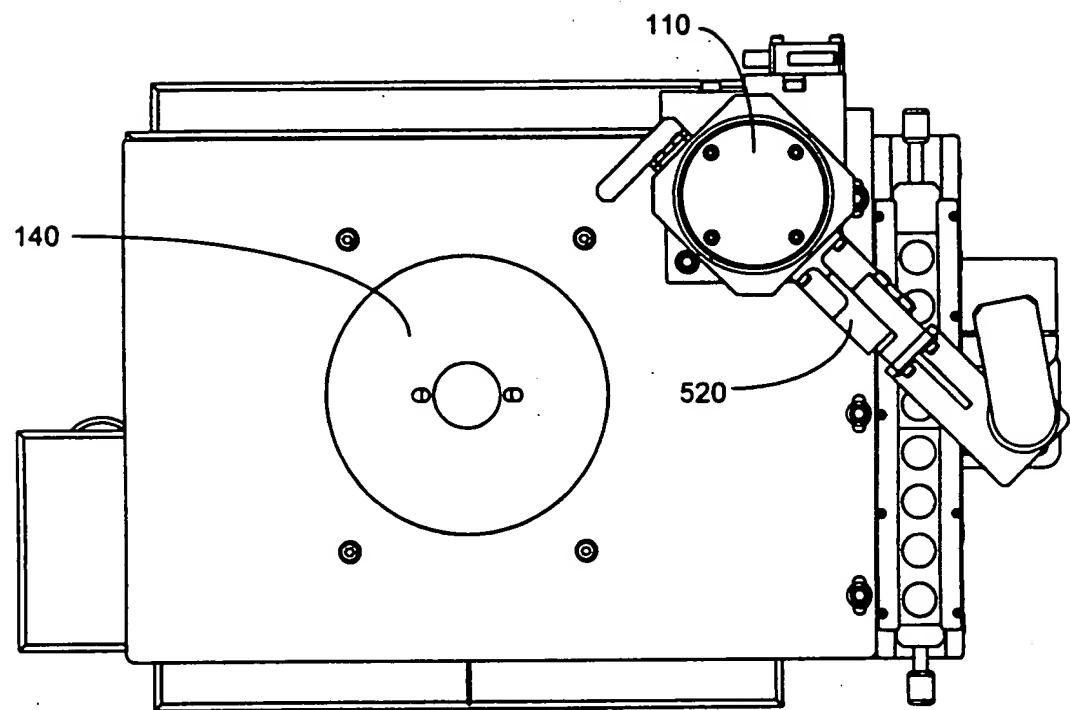


FIG. 11

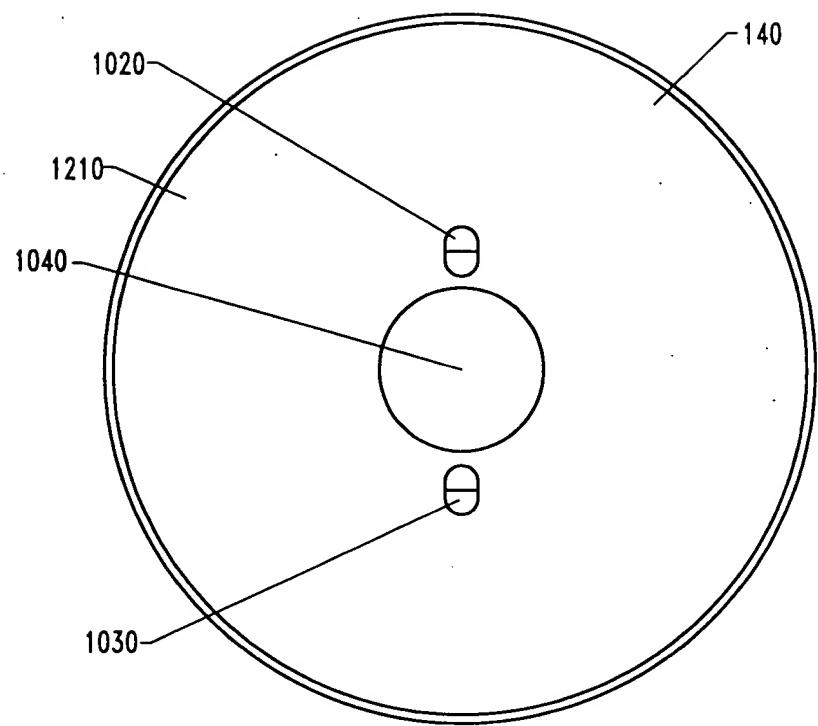


FIG. 12

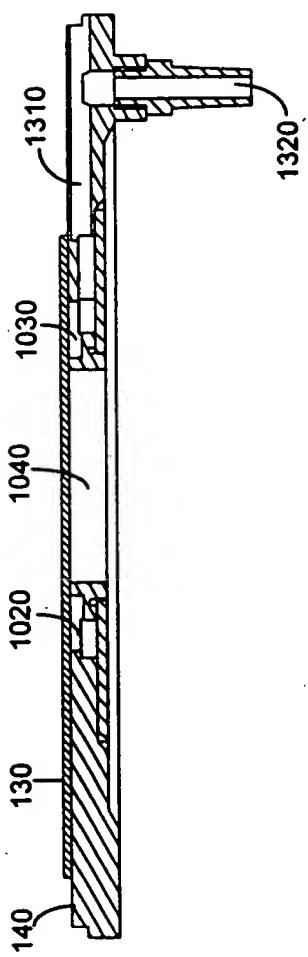


FIG. 13

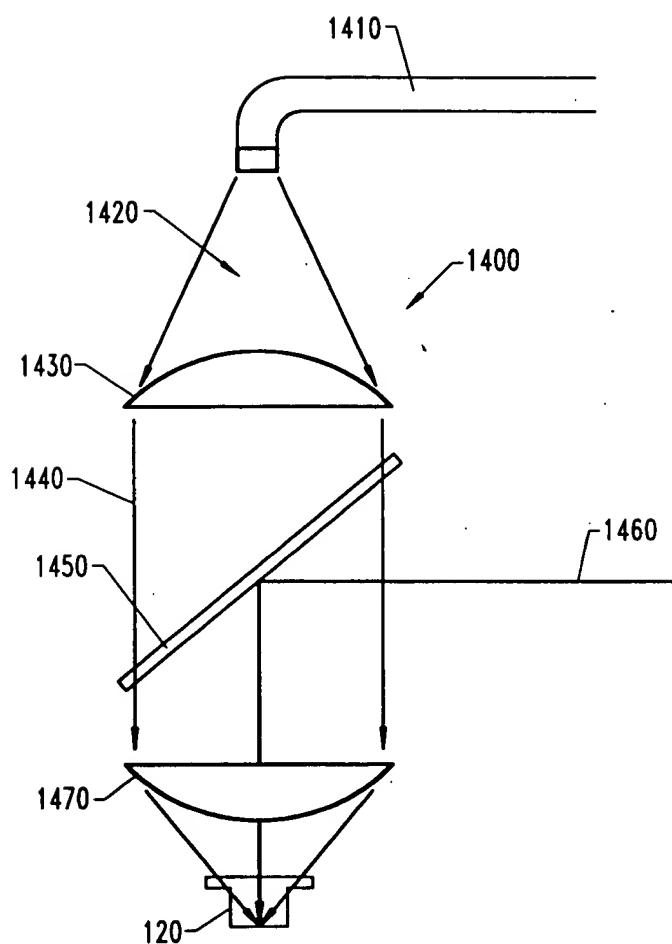


FIG. 14

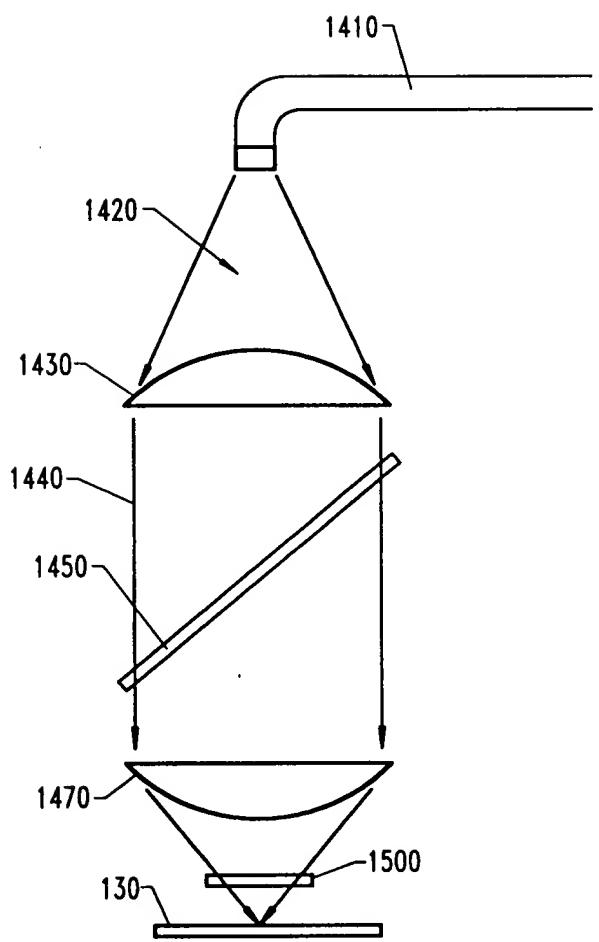


FIG. 15

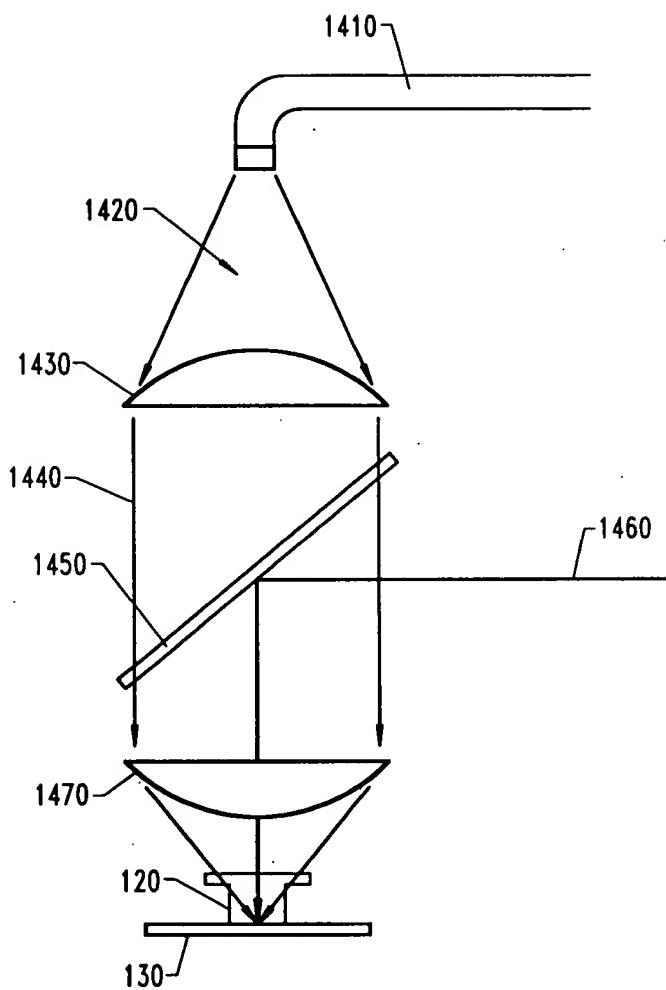


FIG. 16

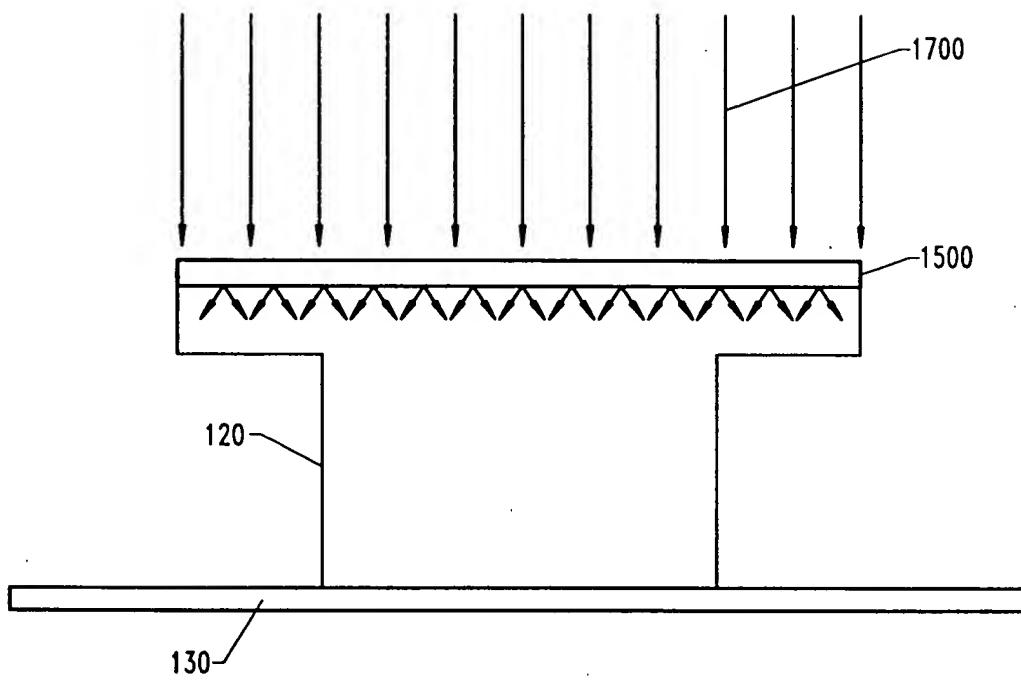


FIG. 17

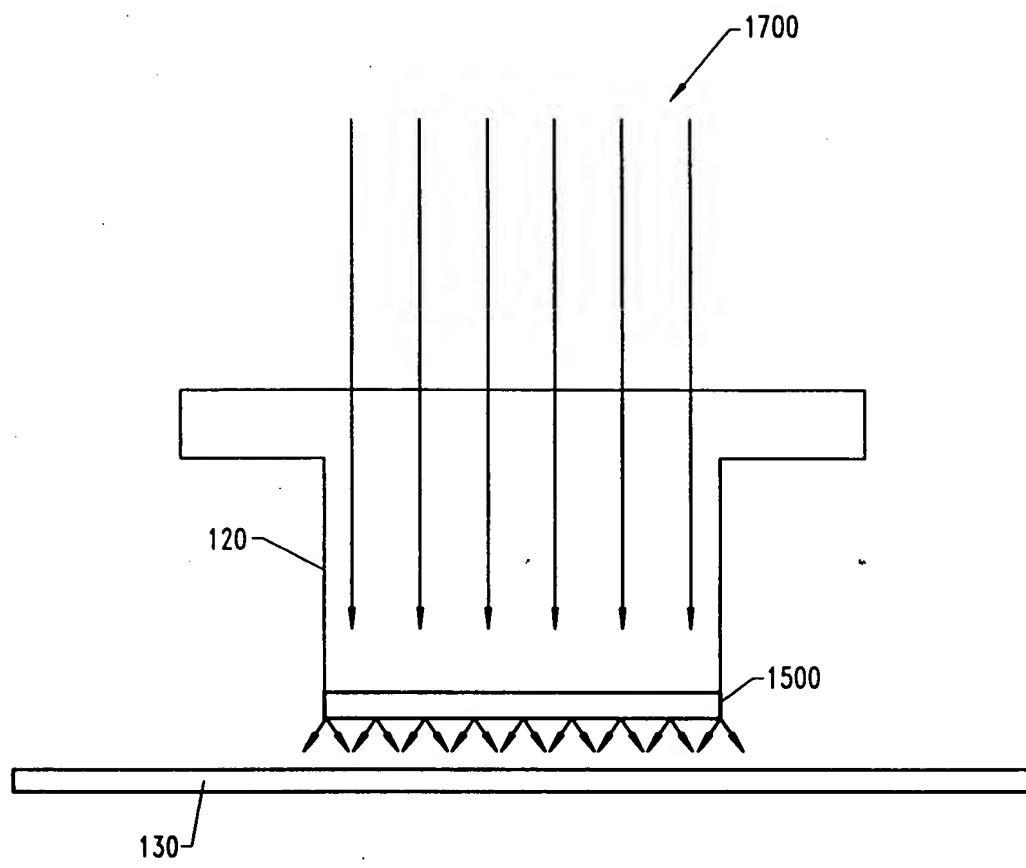


FIG. 18

09/208,604

UNITED STATES PATENT APPLICATION
ENTITLED

**SMALL DIAMETER LASER CAPTURE
MICRODISSECTION**

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David F. Head, and Christopher E. Todd

Citizenship: All of the United States

PATENT
Attorney Docket No. 17726-717

SMALL DIAMETER LASER CAPTURE MICRODISSECTION

Inventors: Thomas M. Baer; Mark A. Enright
David F. Head; and Christopher E. Todd

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is, under 35 U.S.C. § 120, a continuation-in-part of U.S. Ser. No. 09/018,452, filed February 4, 1998, now pending, which is in turn a continuation-in-part of both U.S. Ser. No. 60/060,731, filed October 1, 1997, now pending, and U.S. Ser. No. 60/037,864, filed February 7, 1997, now abandoned, the entire contents of all which are hereby incorporated herein by reference as if fully set forth herein.

10

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to the field of laser capture microdissection (LCM). More particularly, the invention relates to inverted microscopes that include specialized apparatus for performing LCM. Specifically, a preferred implementation of the invention relates to an inverted microscope that includes a cap handling subsystem, an illumination/laser optical subsystem, a vacuum chuck subsystem, and a manual joystick subsystem. The invention thus relates to inverted

microscopes of the type that can be termed laser capture microdissection inverted microscopes.

Discussion of the Related Art

5

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

10

A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

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In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the tissue section, the operator centers them in a target area of the microscope

field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research. For instance, the National Cancer Institute's Cancer Genome Anatomy

Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

5 The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected
10 human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.
15

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue
20
25 is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

5 The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared napthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is
10 mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

15 While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

20 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for
laser capture microdissection. There is also a particular need for an
5 improved method of laser capture microdissection.

A first aspect of the invention is implemented in an embodiment that
is based on a laser capture microdissection method, comprising: providing a
sample that is to undergo laser capture microdissection; positioning said
sample within an optical axis of a laser capture microdissection instrument;
10 providing a transfer film carrier having a substrate surface and a laser
capture microdissection transfer film coupled to said substrate surface;
placing said laser capture microdissection transfer film in juxtaposition with
said sample with a pressure sufficient to allow laser capture microdissection
transfer of a portion of said sample to said laser capture microdissection
transfer film, without forcing nonspecific transfer of a remainder of said
15 sample to said laser capture microdissection film; and then transferring a
portion of said sample to said laser capture microdissection transfer film,
without forcing nonspecific transfer of a remainder of said sample to said
laser capture microdissection transfer film.

20 A second aspect of the invention is implemented in an embodiment
that is based on a laser capture microdissection instrument, comprising: an
inverted microscope including: an illumination/laser optical subsystem; a
translation stage coupled to said illuminator/laser optical subsystem; a cap
handling subsystem coupled to said translation stage; a vacuum chuck
25 subsystem coupled to said translation stage; and a manual joystick
subsystem coupled to said translation stage.

These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

10

BRIEF DESCRIPTION OF THE DRAWINGS

A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

20

25

FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention.

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1.

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention.

5 FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention.

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention.

10 FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention.

FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6.

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention.

15 FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8.

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention.

20 FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10.

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention.

25 FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention.

FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention.

5 FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention.

FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention.

10 FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

15 FIG. 19 illustrates available energy as a function of perpendicular distance from the optical axis of a 5 micron diameter laser beam and a 30 micron diameter laser beam, representing embodiments of the invention.

20 FIG. 20 illustrates a geometric plot of three isotherms within an LCM transfer film corresponding to three different laser beam pulse durations each of which delivers a different total amount of energy, representing embodiments of the invention.

FIG. 21 illustrates a geometric plot of three isotherms within an LCM transfer film corresponding to three different laser beam pulse durations each of which delivers a substantially constant total amount of energy, representing embodiments of the invention.

25 FIG. 22 illustrates a schematic isometric view of a focusing lens subassembly representing an embodiment of the invention.

FIG. 23 illustrates a schematic isometric view of the focusing lens subsystem mounted on an optics plate of an inverted microscope, representing an embodiment of the invention.

5 FIG. 24 illustrates a schematic perspective view of the apparatus shown in FIG. 23 with a focusing lens position control in a first position, representing an embodiment of the invention.

FIG. 25 illustrates another schematic perspective view of the apparatus shown in FIGS. 23-24 with the focusing lens position control in a second position, representing an embodiment of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting 5 embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

10 The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997 entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if 15 fully set forth herein.

20 Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

25 A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables

on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement.

Further, this simultaneous movement can be effected with in a single handed manner. The acquisition of samples is thus made quicker.

5 Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a
10 function of operator hand movement.

15 In addition, the joystick provides tactile feedback not available with electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X and Y) as a function of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

20 Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185.
25 After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic

that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the
5 beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.

Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can
10 be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be appreciated from FIG. 2B that the illumination beam path includes delivery of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

15 Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can
20 reflect and/or absorb the energy from the laser beam.
25

The position of the laser beam path 310 with respect to the portion of
the sample that is to be acquired by the microcentrifuge tube cap 120 can be
seen by an operator via the image acquisition system 230 (not shown in
FIG. 3), which can include a camera. In idle mode, the laser beam path 310
provides a visible low amplitude signal that can be detected via the
acquisition system 230. In pulse mode, the laser beam path 310 delivers
energy to the microcentrifuge tube cap 120 and the optical characteristics of
the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that
substantially none of the energy from the laser beam exits through ocular
370.

Suitable laser pulse widths are from 0 to approximately 1 second,
preferably from 0 to approximately 100 milliseconds, more preferably
approximately 50 milliseconds. In a preferred embodiment the wavelength
of the laser is 810 nanometers. In a preferred embodiment the spot size of
the laser at the EVA material located on microcentrifuge tube cap 120 is
variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more
preferably from 5 to 30 microns. These ranges are relatively preferred when
designing the optical subsystem. From the standpoint of the clinical
operator, the widest spot size range is the most versatile. A lower end point
in the spot size range on the order of 5 microns is useful for transferring
single cells.

Suitable lasers can be selected from a wide power range. For
example, a 100 watt laser can be used. On the other hand, a 50 mW laser
can be used. The laser can be connected to the rest of the optical subsystem
with a fiber optical coupling. Smaller spot sizes are obtainable using

diffraction limited laser diodes and/or single mode fiber optics. Single mode fiber allows a diffraction limited beam.

While the laser diode can be run in a standard mode such as TEM₀₀, other intensity profiles can be used for different types of applications.

5 Further, the beam diameter could be changed with a stepped lens instead of lens 350.

10 Changing the beam diameter permits the size of the portion of the sample that is acquired to be adjusted. Given a tightly focused initial condition, the beam size can be increased by defocusing. Given a defocused initial condition, the beam size can be decreased by focusing. The change in focus can be in fixed amounts. The change in focus can be obtained by means of indents on a movable lens mounting and/or by means of optical glass steps. In any event, increasing/decreasing the optical path length is the effect that is needed to alter the focus of the beam, thereby altering the spot size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

15 Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one

member selected from the group consisting of chromophores and
fluorescent dyes (synthetic or organic), and, the process of operating the
instrument includes identifying at least a portion of said sample with light
that excites the at least one member, before the step of transferring said
portion of said sample to said laser capture microdissection transfer film.

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Still referring to FIG. 4, the beam 420 is reflected by a mirror 430.
The beam 420 is then reflected by the dichroic mirror 340. In this way the
beam 420 can be made coincident with both the laser beam path 310 and the
white light from illuminator 170. It should be noted that the beam 420 and
the laser beam path 310 are shown in a spaced-apart configuration for clarity
only. The beam 420 and the laser beam path 310 can be coaxial.
Fluorescence emitted by the sample beneath the microcentrifuge tube cap
120 then travels through the objective lens 360 to be viewed by the operator
through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling
mechanic subassembly 110 is depicted. The cap handling mechanic
subassembly 110 includes a dampener 510. The dampener 510 is a structure
for damping vertical motion of the cap handling mechanic subassembly 110.
The dampener 510 is adapted to lower the microcentrifuge tube cap 120
down towards the translation stage in a reproducible manner. The dampener
510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g.,
hydraulic tube) or any other dynamic structure capable of retarding the
vertical motion of the subassembly 110 so as not to generate an impulse. As
the microcentrifuge tube cap 120 contacts the slide on which the sample
rests (not shown), the working end 112 of an arm 520 that is coupled to the
dampener 510 continues downward at a reproducible rate. Therefore, the

top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed
5 against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the
10 microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 is picked up off of the slide. The dampener within the mechanism acts as a dash pot to control the
15 velocity of the pickup arm.

The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel
20 to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of
25 the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping

the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 off the slide (not shown).

The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture microdissection transfer film is approximately 0.26 square inches.

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be

appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to the position depicted in FIGS. 6-7).

Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum

chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

10 The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

15 The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

20 There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place. Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

25 Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass

slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430 collimates the light from the fiber optic 1410. The collimator lens 1430 can be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens).
A collimated beam 1440 from the collimator lens 1430 then passes though a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460 is coaxial with the white light illumination. Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG 010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470 can have a numerical aperture of approximately 0.8. This can be characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that contains the cells to be sampled.

5 More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type eliminates the need for refractive index matching of the sample. Such a
10 scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk or opal glass which is
15 a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example, frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser.
20 In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light sufficiently.

25 Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the

image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed into the microcentrifuge tube cap 120 so as to acquire the desired sample.

The purpose of the illuminator design is to provide a very high
5 numerical aperture illuminator for an LCM device. Such an LCM device
requires a large working distance. While an illuminator that uses a 40X
objective with 0.8 numerical aperture may seem to give better visualization,
this design has problems since the working distance for the 40X objective is
very small, (e.g., less than 1 millimeter). Thus it is critical for a design that
10 uses a thick dome carrier to have an illumination design with a much longer
working distance. A thick dome carrier is a sample carrier whose top and
bottom are spaced apart more than a small distance. This is important
because the sample is adjacent the bottom of the sample carrier and the
objective cannot move closer to the sample than the top of the sample
15 carrier.

The focusing lens 190 can be replaced with a Melles Griot aspheric
condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture
of about 0.75 and a working distance of about 25 millimeters. Such a lens is
not corrected for chromatic aberrations like the 40X objective. Experiments
20 done using a spherical lens as a condenser gave good improvement in
visualization. This spherical lens clearly did not have the corrections for
aberrations that are built into the 40X objective.

The laser beam can be focused through this condenser lens like the
focusing lens 190. This condenser lens has roughly one-half the focal
25 length of the current lens so the laser beam will be focused down to roughly
15 microns.

In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

5 Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality 10 of the imagery is improved.

15 The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

20 Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

25 The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or

the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

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The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the velocity should be such that the sample is thixotropically sheared.

Small Spot Size Laser Capture Microdissection

The invention includes a method for microdissecting small regions of tissue samples. The invention also includes equipment adapted to facilitate laser capture microdissection of small tissue regions. Tissue regions with diameters under approximately 20 microns, approximately 10 microns, approximately 5 microns and/or approximately 1 micron in diameter can be defined as small. Such small tissue regions require small spot sizes to be laser capture microdissected.

In the past, the original method for microdissection using laser capture microdissection (LCM) employed a freestanding film that was applied to the surface of the tissue by gently pressing the film onto the sample. The film above the tissue section of interest was then heated and melted by a several hundred-millisecond (msec.) pulse from a CO₂ laser. The length of the laser pulse was chosen so as to allow the film to reach thermal equilibrium during the laser pulse, melting the film over the laser exposure region, but allowing lower laser powers to be used and minimizing the peak temperature experienced by the tissue, thereby preventing damage to DNA/RNA and/or proteins in the sample. This original method avoided short pulse since there appeared to be no advantage to shorter pulses for melting the film and the shorter pulses exposed the tissue to higher laser fluences, thereby potentially damaging the sample.

In general, the size of the transferred tissue region is determined by the diameter of the portion of the LCM transfer film that is raised to a temperature at which the LCM transfer film becomes hot enough to melt and fuse with the tissue sample. For long pulses, the transfer size is

determined primarily by the power of the laser beam and by the thermal characteristics (e.g., thermal capacity and diffusion) of the LCM transfer film. For pulse widths of from approximately 50 msec. to approximately 250 msec., laser beam power of approximately 50 mW, beam diameter of approximately 50-100 microns, and film thickness of approximately 100 microns, the tissue transfer size is roughly the same size as the laser beam diameter. As the power is increased, the transfer size increases. However, for pulse widths of from approximately 50 msec. to approximately 250 msec., the diameter of the transferred spot does not vary as a function of the pulse duration.

As discussed above with regard to the invention, the LCM transfer film can be attached to a flat, rigid carrier rather than being used as a free standing film. This approach offers several advantages. When the LCM transfer film is mounted on a rigid film carrier, the transfer film is injected into the tissue when the laser heats the transfer film. In more detail, when the transfer film is exposed to the laser beam, the film in the exposed region expands, pushing against the rigid film carrier and against the surrounding unheated portions of the LCM transfer film. This expansion forces the melted transfer film downwards and into the sample tissue. Thus, the LCM transfer film need not be in direct contact with the sample tissue. The heated portion of the transfer film reaches out during the laser pulse, fuses with the tissue, and then the tissue is captured within the perimeter of that portion of the transfer film that is exposed to the laser beam.

With a 40 micron thick film doped with roughly 0.1% by weight infrared absorbing dye (e.g., vanadyl phthalocyanine, Aldrich #CA 105011-00-5) mounted on a flat plexiglass substrate, and using a laser diode tuned to

the peak of the absorption bands of the dye, with a pulse width of 50 msec., a laser power of approximately 30 mW, and a beam size of approximately 30 microns, the transferred tissue region is roughly the same size as the laser beam. Further, for these parameters, the transfer spot size diameter
5 increases as the laser power is increased, but the diameter of the transfer spot size does not increase substantially as the pulse duration (aka pulse width) is increased. Significantly, as the laser spot size is reduced below approximately 20 microns, the transfer size does not get smaller, for a pulse duration of approximately 50 msec., or longer.

10 An important aspect of the invention is to provide a method that allows the acquisition of an LCM transfer region of less than approximately 30 microns, preferably less than approximately 20 microns, more preferably less than approximately 10 microns, even less than approximately 5 microns. It has been found that a reliable method for obtaining smaller transfer spot sizes (e.g., less than approximately 10 microns in diameter)
15 includes reducing the pulse width of the laser to less than 5 msec., preferably less than approximately 1 msec., even less than approximately 0.5 msec. and simultaneously increasing the peak power of the laser to at least approximately 50 mW. Significantly, these pulse widths and powers do not damage the macromolecules in tissue samples.
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At longer pulse widths, it is believed that the exposed region of the LCM transfer film reaches thermal equilibrium. In these circumstances the width of the transfer region is defined roughly by the diameter of the film region that has a temperature hot enough at thermal equilibrium to melt the
25 film and fuse the film to the tissue. Since the film region excited by the laser has reached thermal equilibrium with the laser operating in the long

pulse width regime, the size of the transfer region is substantially independent of the pulse width of the laser pulse. Moreover, if the laser spot size is reduced below roughly 20 microns, the diameter of the melted region of the film at thermal equilibrium remains approximately 20-30

5 microns. Thus, even at smaller beam diameters, the transfer size remains 20-30 microns since this is the size of the heated region at equilibrium.

However, at pulse widths below approximately 1 msec., the film does not reach thermal equilibrium and the film in the region exposed to the laser beam heats up quickly, expanding to contact, and fuse, with the tissue.

10 For a short pulse (e.g., less than approximately 1 msec.) there is insufficient time for the temperature of the film outside the region exposed to the laser pulse to increase since the laser pulse is so short. The region of the film outside the exposed area remains below the melting point and does not fuse to the tissue. Thus, for short pulses the transfer region size is not increased

15 by thermal diffusion. For short pulses, the transfer size is determined primarily by the laser spot diameter. For short pulses, the transfer spot size increases as the pulse width of the laser is increased. This is in contrast to the longer pulse regime, indicating operation in a different thermal diffusion regime.

20 Previous embodiments of LCM (using CO₂ or diode lasers and an LCM transfer film that is not mounted to a film carrier) taught against the use of short pulses since short pulses required an increase in the laser fluence experienced by the tissue sample. It was thought that this fluence should be minimized to minimize tissue damage. Moreover, at spot sizes

25 above approximately 50 microns and film thicknesses of from 50 to 100 microns, no advantage was observed in going to very short pulses.

It is surprising that short pulse durations provide sufficient time for
the film to expand and contact the tissue. It is also surprising that short
pulse widths allow sufficient time to melt the film and fuse the film to the
tissue. The discovery by the inventors that reliable LCM at short pulse
widths is even possible is a most surprising result and it is even more
surprising that small transfer diameters require short pulse excitation. In
general, whenever the diameter of the laser beam becomes substantially
smaller than the thickness of the LCM transfer film, it may be important to
implement the LCM process using short pulses in order to avoid thermal
diffusion and subsequent broadening of the transfer spot size.

Turning to FIG. 19, two spatial domain energy profiles delivered to
the LCM transfer film (e.g., EVA) by two different laser beam diameters are
depicted. These energy profiles display energy (E) as a function of
perpendicular distance from the optical axis (DIA) of the beam. A 5 micron
diameter laser beam energy profile is depicted with a solid line in FIG. 19.
A 30 micron diameter laser beam energy profile is depicted with a dashed
line in FIG. 19. These two profiles illustrate the comparison of the size of
the energy profile delivered to the EVA by the laser and the approximate
size of the resultant transfer spot. It can be appreciated that the profiles
shown in FIG. 19 are coaxial and symmetric with regard to a spatial position
corresponding to the center of the laser beams.

Turning now to FIG. 20, a two dimensional geometric plot of three
isotherms within an LCM transfer film 2000 produced by a constant laser
beam diameter generated in long pulse mode are depicted. In FIG. 20, the
melt depth with a 30 micron spot size is shown. The upper horizontal line
represents a tissue/film interface 2010. The lower horizontal line represents

a cap/film interface 2020. The area inside a given curve represents that portion of the transfer film that is in a viscous (e.g. liquid) state. It can be appreciated that the three profiles shown in FIG. 20 are coaxial and symmetric with regard to a spatial position that corresponds to the center of the laser beam. The innermost profile represents a set of conditions T1 (time), E1 (energy), the middle profile represents conditions T2, E2, and the outermost profile represents conditions T3, E3, where $T_1 < T_2 < T_3$ and $E_1 < E_2 < E_3$.

The pulses depicted in FIG. 20 are sufficiently long so that heat diffusion is substantially isotropic. Due to this, the transfer spot size change is only weakly dependent on change in the energy delivered. The added energy is provided by holding the power constant and increasing the pulse duration. In this long pulse regime, the added energy is simply diffused to the outer transfer film material and contributes very little to any change in spot size.

Turning now to FIG. 21, a two dimensional geometric plot of three isotherms within the LCM transfer film 2000 produced by a constant laser beam diameter generated in short pulse mode are depicted. In FIG. 21, the melt depth with a 5 micron beam spot size is shown. Again, the upper horizontal line represents the tissue/film interface 2010 and the lower horizontal line represents the cap/film interface 2020. It can be appreciated that the profiles shown in FIG. 21 are coaxial and symmetric with regard to a spatial position that corresponds to the center of the beam. The innermost profile represents a set of conditions T1 (time), E1 (energy), the middle profile represents another set of conditions T2, E2, and the outermost profile represents yet another set of conditions T3, E3, where $T_1 < T_2 < T_3$ and

E₁=E₂=E₃. Thus, the power at T₁ is greater than the power at T₂, and the power at both T₁ and T₂ are greater than the power at T₃.

The cross over point (protrusion) for the T₁, E₁ curve above the tissue/film interface 2010 shows the transfer spot size under these 5 conditions. It can be appreciated that each curve has the same total energy, but the T₁, E₁ curve has high enough power and short enough pulse to breach to the tissue/film interface with liquid EVA (defining the transfer spot size). In all the cases shown in FIG. 21, the time duration is sufficiently short so radial thermal diffusion into the rest of the EVA is 10 minimal (i.e., the heat/energy is anisotropically dissipated, preferentially in the axial direction). Curves T₂ and T₃ have slightly longer pulse durations (and corresponding lower power levels) which permit enough radial heat diffusion so that the transfer film 2000 at the interface 2010 is not heated to its melting point.

15 Turning now to FIG. 22, a focusing lens subassembly 2200 includes a frame 2210 that is mechanically coupled to a lens 2220. The frame 2210 is slidably coupled to a mount 2230 that is adapted to be rigidly connected to an optics plate (not shown in FIG. 22) of an LCM apparatus, such as, for example, an inverted microscope. The frame 2210 can be readily moved 20 along one axis with regard to the mount 2230. This relative movement is depicted by the double-headed arrow in FIG. 22. A ramp 2240 is pivotally coupled to the frame 2210. Pivoting the ramp 2240 permits the inclination of the ramp 2240 to be adjusted with regard to the plane defined by the optical axis of the lens 2220. A stop 2250 is in contact with the ramp 2240 25 and acts to limit the pivoting motion of the ramp 2240 in one direction (i.e.,

toward the frame 2210). The pivoting motion of the ramp 2240 away from the stop 2250 is depicted in FIG. 22 with a single-headed arrow.

Referring now to FIG. 23, the focusing lens subassembly 2200 is shown in mechanical engagement with an optics plate 2310 of an inverted microscope. Specifically, the mount 2230 is bolted to the optics plate 2310. Relative movement between the frame 2210 and the optics plate 2310 is represented in FIG. 23 with a double-headed arrow.

Turning now to FIG. 24, a bracket 2410 is mechanically coupled to the optics plate 2310. A pivoting arm 2420 is pivotally connected to the bracket 2410. The pivoting arm 2420 is mechanically coupled to the ramp 2240 via a contact point (not shown in FIG. 24). A handle 2430 is connected to the pivoting arm 2420. The pivotable arm 2420 can be provided with mechanical detents to give tactile feedback to a human operator, who can angularly displace the handle 2430. A rotatable knob 2440 is mechanically coupled to the pivoting arm 2420. The rotatable knob 2440 permits adjustment of the contact point which moves the ramp 2240, thereby providing a fine focus adjustment for the lens 2220.

Still referring to FIG. 24, angular deflection of the handle 2430 causes the pivoting arm 2420 to rotate with regard to the bracket 2410, thereby causing the contact point to be moved along the ramp 2240. Gravity can keep the inclined surface of the ramp 2240 adjacent to the contact point. Thus, movement of the contact point across the inclined plane surface of the ramp 2240 causes the frame 2210 (and therefore, the lens 2220) to be moved with regard to the optics plate 2310.

Referring now to FIG. 25, the focusing lens subassembly 2200 and the optic plate 2310 are shown from a slightly different perspective so that

the contact point 2510 is visible. But more importantly, in FIG. 25, the handle 2430, the pivoting arm 2420, and the rotatable knob 2440 have been angularly deflected with regard to the bracket 2410 compared to their position as depicted in FIG. 24. Referring to FIG. 25, it can be appreciated
5 that the contact point 2510 has been displaced to a different point on inclined surface of the ramp 2240, compared to the position of the contact point 2510 in FIG. 24. Specifically, with regard to the frame 2210, the contact point 2510 has been repositioned to a lower point of the ramp 2240 in FIG. 25. The position of the contact point 2510 in FIG. 25 can
10 correspond to a large spot size diameter while the position of the contact point in FIG. 24 can correspond to a small spot size diameter. Of course, an intermediate spot size diameter can be defined by an intermediate position of the pivotable arm 2420 that is between the positions shown in FIG. 24 and FIG. 25.

15 Operation of the LCM apparatus can be made more convenient and more rapid by providing equipment that automatically adjusts the pulse duration and laser beam power as a function of the position of the pivotable arm 2420. This allows the operator to concentrate on choosing the optimum spot size while the apparatus automatically adjusts the pulse duration and
20 laser beam power.

For example, the position shown in FIG. 24 of the pivotable arm 2420 can correspond to a spot size diameter of from approximately 5 microns to approximately 7 microns. When the apparatus is positioned into the configuration shown in FIG. 24, the pulse width duration can be
25 automatically set to within a range of from approximately 100 microseconds to approximately 2 milliseconds, preferably less than 1 millisecond.

Similarly, the power of the beam can be automatically adjusted to a range of from approximately 10 milliwatts to approximately 100 milliwatts. These automatic settings can be achieved with a multiposition switch that is incorporated into the pivoting arm 2420 in combination with a look-up table stored in a memory. In preferred embodiments, the look-up table can be (re)programed by a human operator, or other outside data source.

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As another example, the position of the pivotable arm 2420 in FIG. 25 can correspond to a spot size diameter of approximately 30 microns.

When the apparatus is positioned into the configuration shown in FIG. 25, the pulse duration can be automatically adjusted to a value of from approximately 20 milliseconds to approximately 60 milliseconds.

Similarly, in this position, the power of the laser beam can be automatically adjusted to within a range of from approximately 20 milliwatts to approximately 100 milliwatts.

In this way, a human operator can select a spot size diameter by angular displacement of the handle 2430 with an easy movement of their left hand, while the pulse duration and power are automatically readjusted. Specific angular positions of the pivotable arm 2420 that result in automatic adjustment of the pulse duration, beam power, and/or other operational parameters of the LCM apparatus can be termed opto-interrupts. Of course, the specific spot size diameters, pulse durations, and beam powers given in the two examples above are merely illustrative. Preferred embodiments of the invention can permit the operator, or other programmer, to redefine the parameter envelopes associated with specific angular positions of the handle 2430, the pivotable arm 2420 and the rotatable knob 2440. The envelope

endpoints and other operational parameters can be stored in the memory of a computing device.

Practical Applications of the Invention

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A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases. This database will be used to more fully understand that pathogenesis of 10 cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce 15 patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually innumerable uses for the invention, all of which need not be detailed here.

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Advantages of the Invention

A laser capture microdissection instrument and/or method representing an embodiment of the invention can be cost effective and 25 advantageous for at least the following reasons. The invention will replace current methods with better technology that allows for more accurate and

reproducible results. The invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

5 All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent was specifically and individually indicated to be incorporated in its entirety by reference.

10 All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the
15 features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

For example, the individual components need not be formed in the
20 disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein
25 is described as a physically separate module, it will be manifest that the LCM instrument may be integrated into other apparatus with which it is

associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

- 5 It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements.
- 10 The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
providing a sample that is to undergo laser capture microdissection;
positioning said sample within an optical axis of a laser capture
microdissection instrument, said laser capture microdissection instrument
including an illumination/laser beam delivery system;
providing a transfer film carrier having a substrate surface and a laser
capture microdissection transfer film coupled to said substrate surface;
placing said laser capture microdissection transfer film in
juxtaposition with said sample with a pressure sufficient to allow laser
capture microdissection transfer of a portion of said sample to said laser
capture microdissection transfer film, without forcing nonspecific transfer of
a remainder of said sample to said laser capture microdissection film; then
illuminating said sample with said illumination/laser beam delivery
system; and then
transferring a portion of said sample to said laser capture
microdissection transfer film, without forcing nonspecific transfer of a
remainder of said sample to said laser capture microdissection transfer film,
by delivering energy to said laser capture microdissection transfer film with
a pulse from said illumination/laser beam delivery system, wherein thermal
energy from said pulse is anisotropically dissipated along an axial direction
that is substantially parallel to said optical axis.

2. The laser capture microdissection method of Claim 1, further comprising reducing a spot size defined by said illumination/laser beam delivery system before the step of transferring a portion of said sample to said laser capture microdissection transfer film.
3. The laser capture microdissection method of Claim 2, wherein reducing a spot size includes moving a focusing lens.
4. The laser capture microdissection method of Claim 3, wherein moving a focusing lens includes moving a contact point along a ramp that is mechanically coupled to said focusing lens.
5. The laser capture microdissection method of Claim 4, wherein moving a contact point includes rotating a pivotable arm that is mechanically coupled to said contact point.
6. The laser capture microdissection method of Claim 2, wherein reducing a spot size includes resetting an operational parameter.
7. The laser capture microdissection method of Claim 6, wherein resetting an operational parameter includes resetting a pulse duration of said illumination/laser beam delivery system.
8. The laser capture microdissection method of Claim 6, wherein resetting an operational parameter includes resetting a laser beam power of said illumination/laser beam delivery system.

9. The laser capture microdissection method of Claim 1, further comprising removing said laser capture microdissection film from said sample, after the step of transferring, by moving both said laser capture microdissection transfer film and said portion of said sample away from said remainder of said sample and out of said optical axis with a pivotable transfer arm.

10. The laser capture microdissection method of Claim 1, wherein said sample is provided on a slide and said laser capture microdissection instrument includes a translation stage, and further comprising holding said slide against said translation stage by actuating a vacuum circuit to exert a force between said slide and said translation stage, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

11. A laser capture microdissection instrument, comprising:
an illumination/laser beam delivery system adapted to produce
anisotropic dissipation of thermal energy within a transfer film along an
optical axis of said illumination/laser beam delivery system.

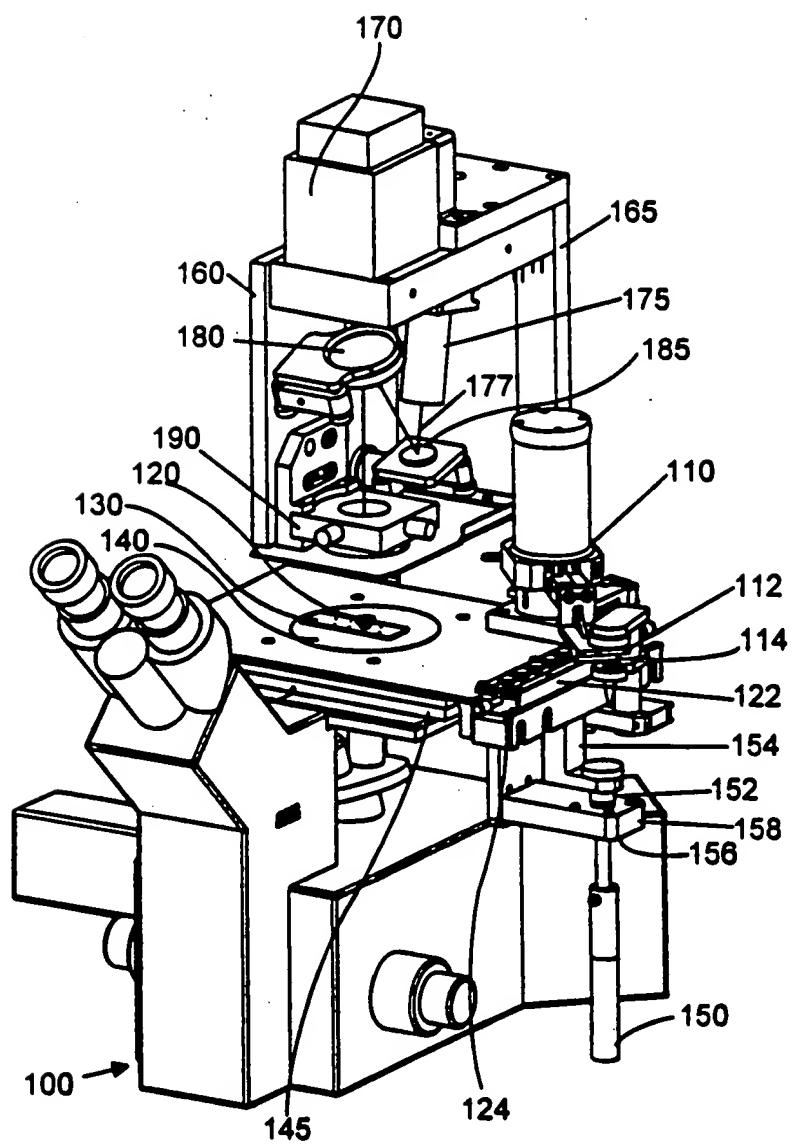
12. The laser capture microdissection instrument of Claim 11, further comprising an automatic adjustment of an operational parameter as a function of a selected spot size.

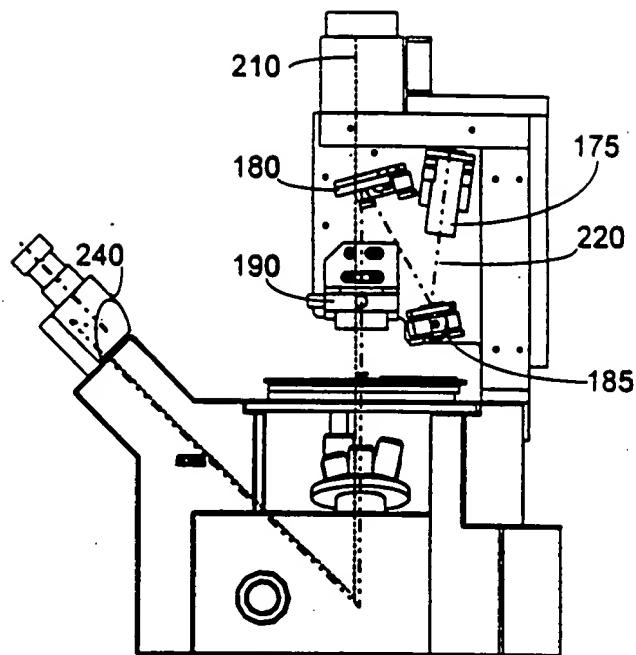
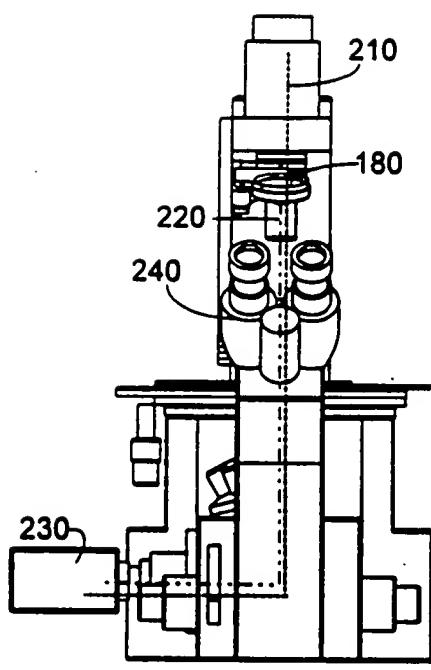
13. The laser capture microdissection instrument of Claim 12, wherein said automatic adjustment of an operational parameter includes automatic adjustment of pulse duration.
14. The laser capture microdissection instrument of Claim 12, wherein said automatic adjustment of an operational parameter includes automatic adjustment of laser beam power.
15. The laser capture microdissection instrument of Claim 11, wherein said illumination/laser beam delivery system includes a variable position focusing lens that is adapted to produce anisotropic dissipation of thermal energy within said transfer film along said optical axis of said illumination/laser beam delivery system.
16. The laser capture microdissection instrument of Claim 15, further comprising a ramp mechanically coupled to said variable position focusing lens.
17. The laser capture microdissection instrument of Claim 16, further comprising a pivotable arm mechanically coupled to said ramp through a contact point.
18. The laser capture microdissection instrument of Claim 11, wherein said laser capture microdissection instrument is an inverted microscope.

19. The laser capture microdissection instrument of Claim 18, further comprising a translation stage, including a vacuum circuit adapted to exert a force between said translation stage and a sample holder upon which a sample that is to undergo laser capture microdissection is positioned.
20. The laser capture microdissection instrument of Claim 18, further comprising a pivotally mounted transfer arm adapted to move laser capture microdissection transfer film carriers into, and out of, said optical axis.

ABSTRACT OF THE DISCLOSURE

Systems and methods for small diameter laser capture
microdissection are disclosed. An inverted microscope adapted for small
diameter laser capture microdissection includes an illumination/laser beam
delivery system with a variable position lens. A method for small diameter
laser capture microdissection includes delivering energy having a pulse
width of approximately 1 mS, or less, and a peak power of approximately 50
mW, or more. The systems and methods provide the advantage of
permitting small regions of tissue samples, for example, under
approximately 10 microns in diameter, to be microdissected.





— WHITE LIGHT ILLUMINATION PATH
— LASER BEAM PATH

FIG. 2A

FIG. 2B

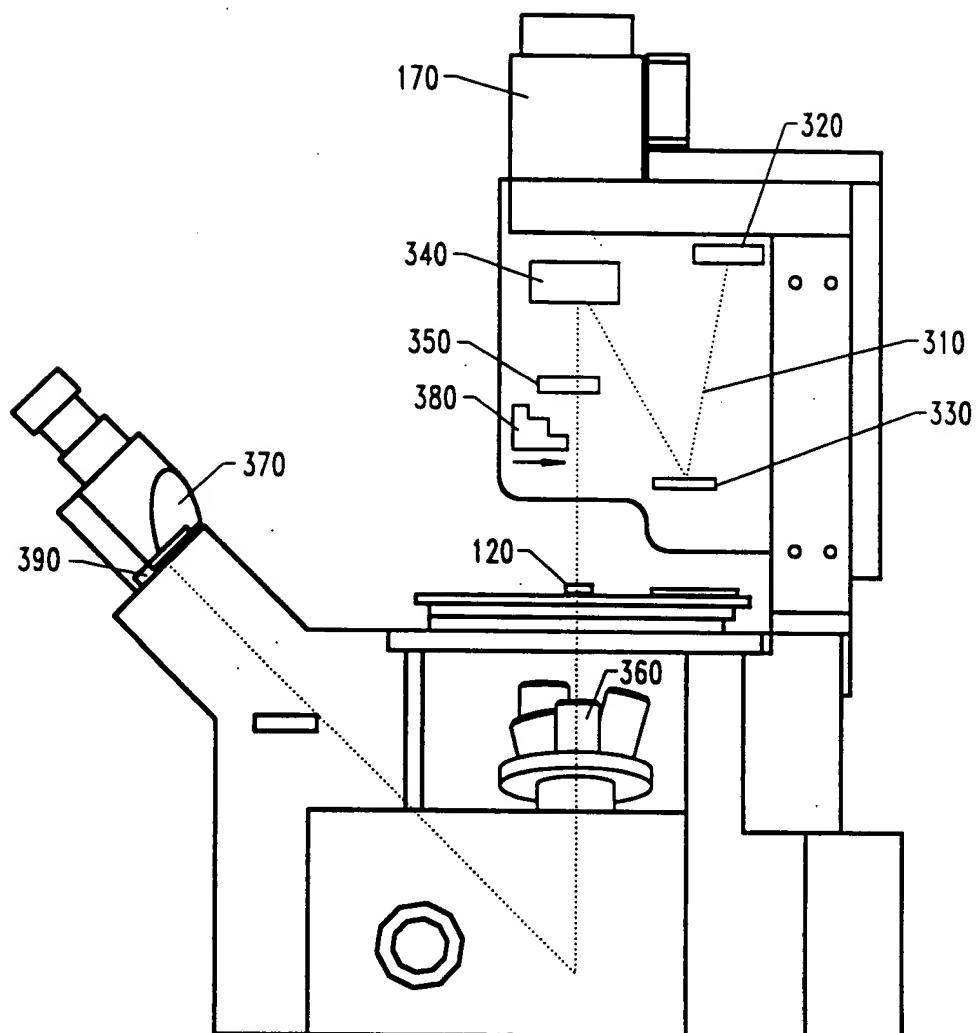


FIG. 3

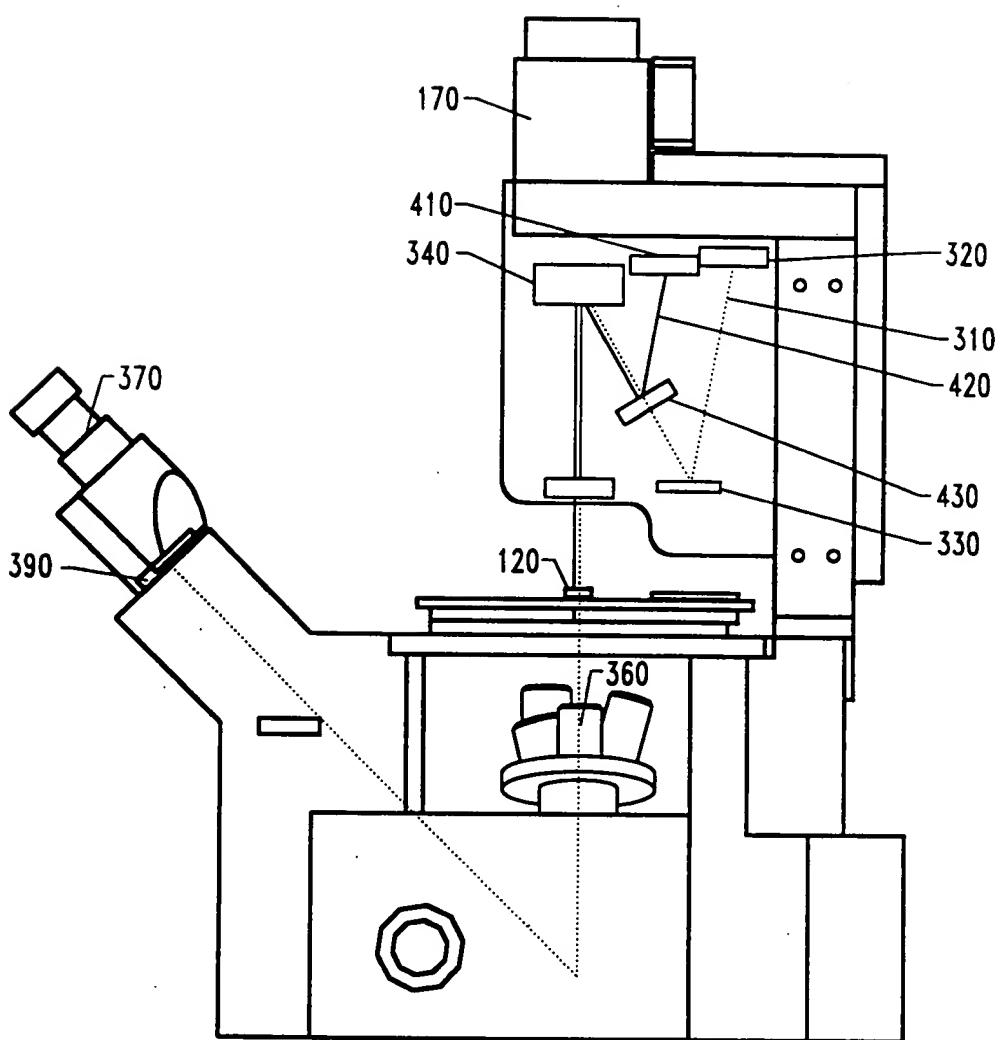


FIG. 4

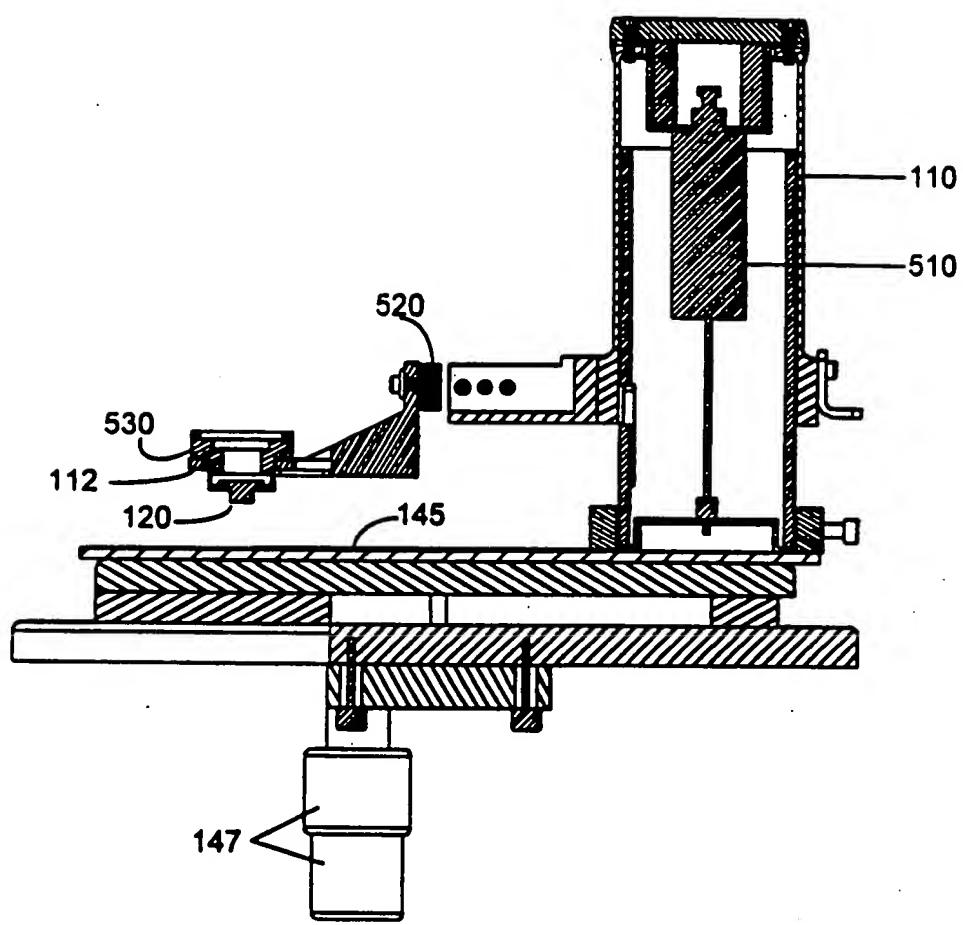


FIG. 5

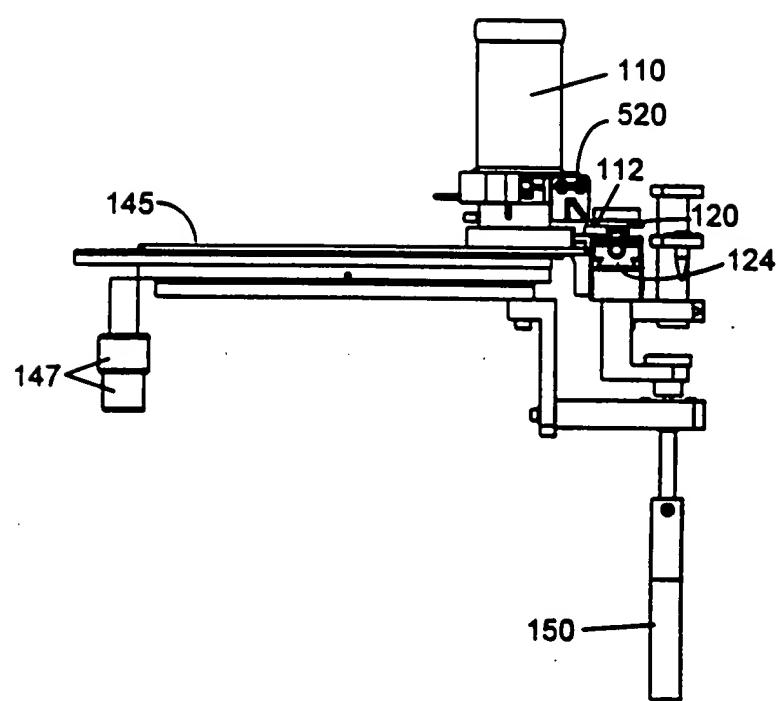


FIG. 6

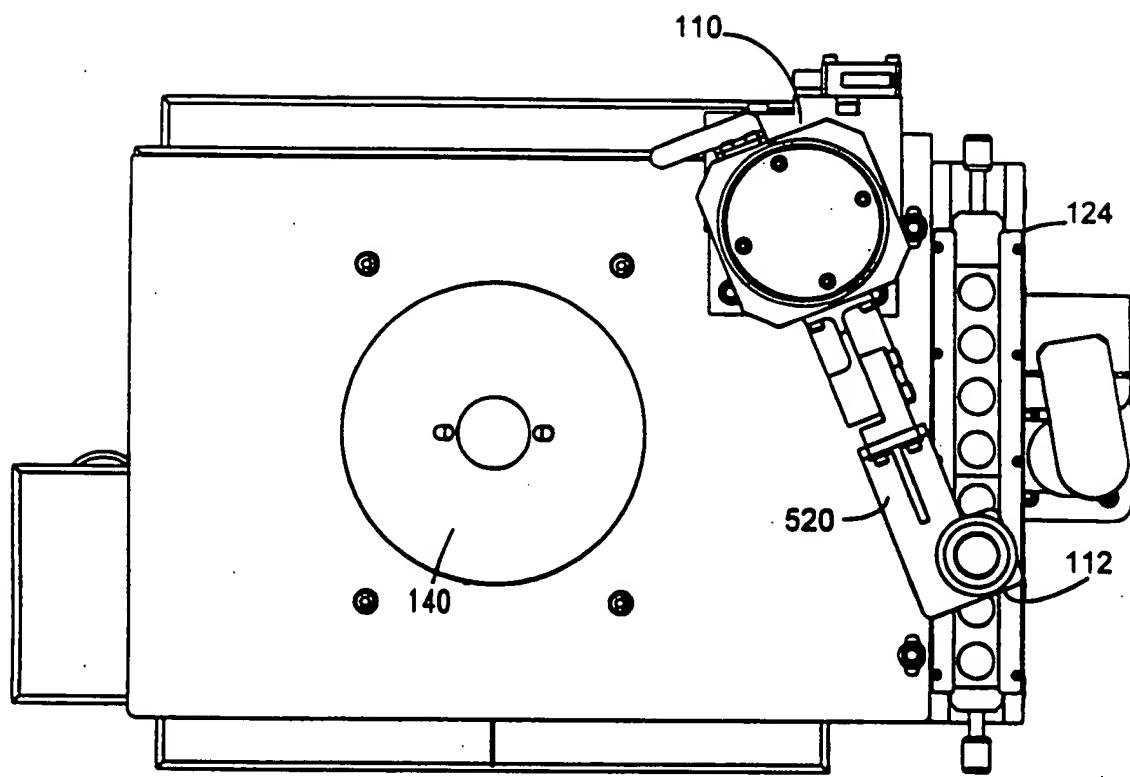


FIG. 7

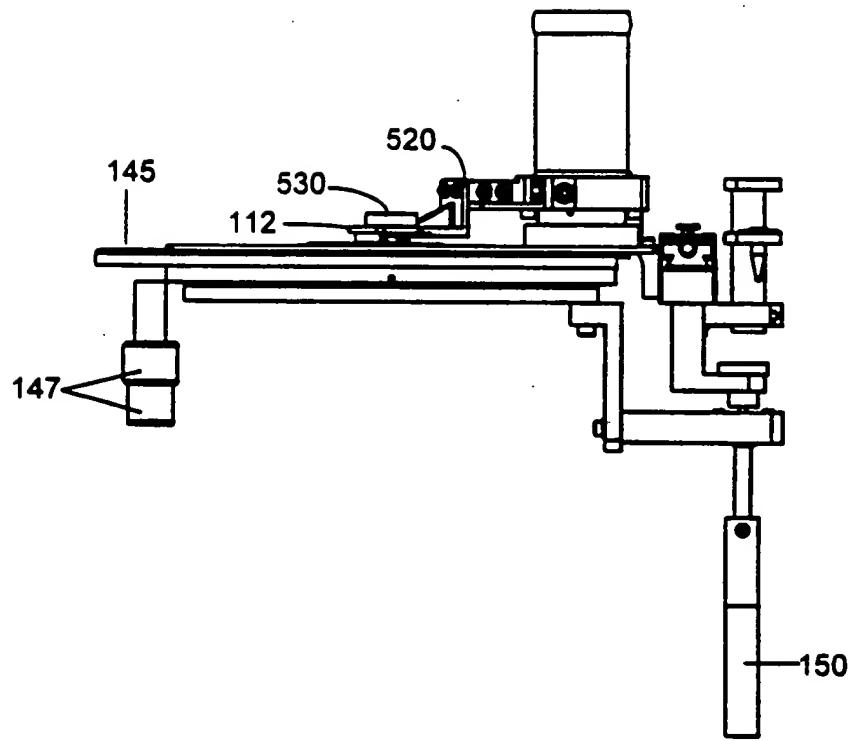


FIG. 8

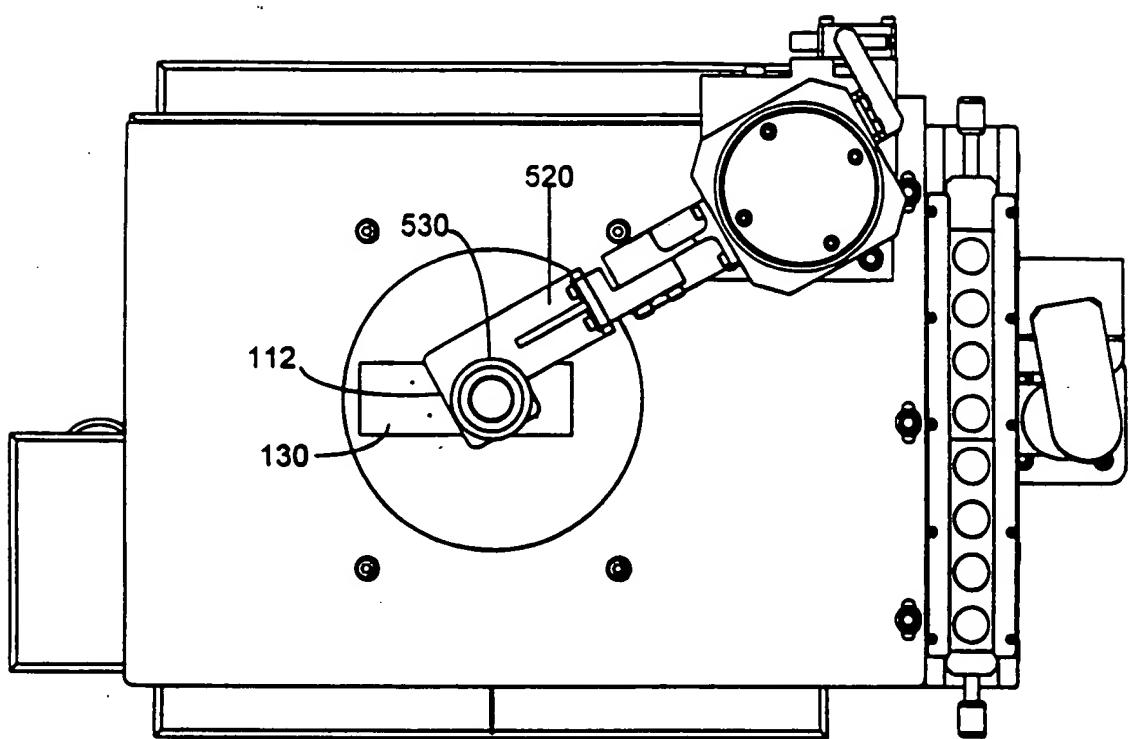


FIG. 9

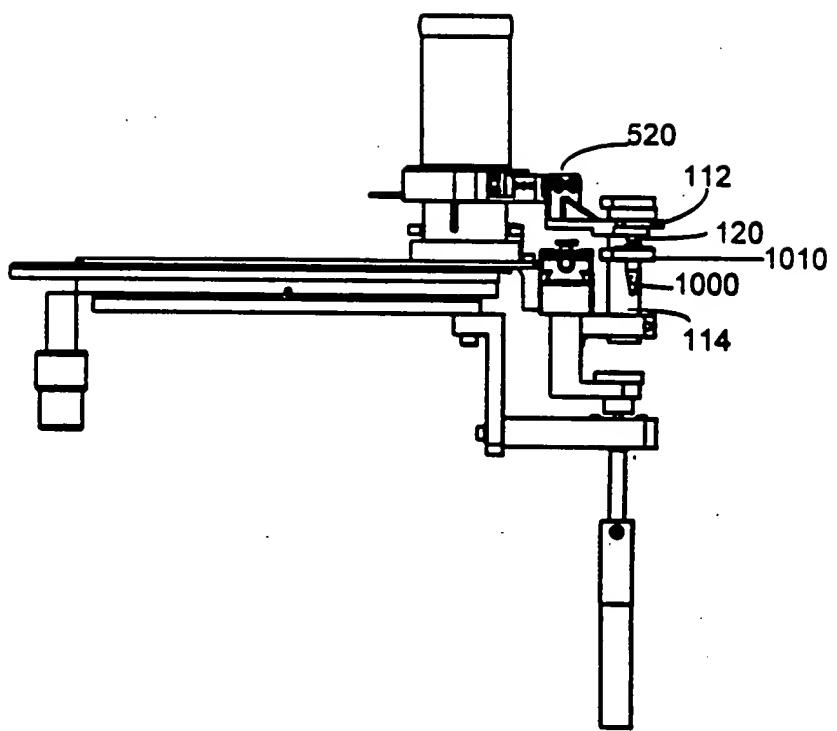


FIG. 10

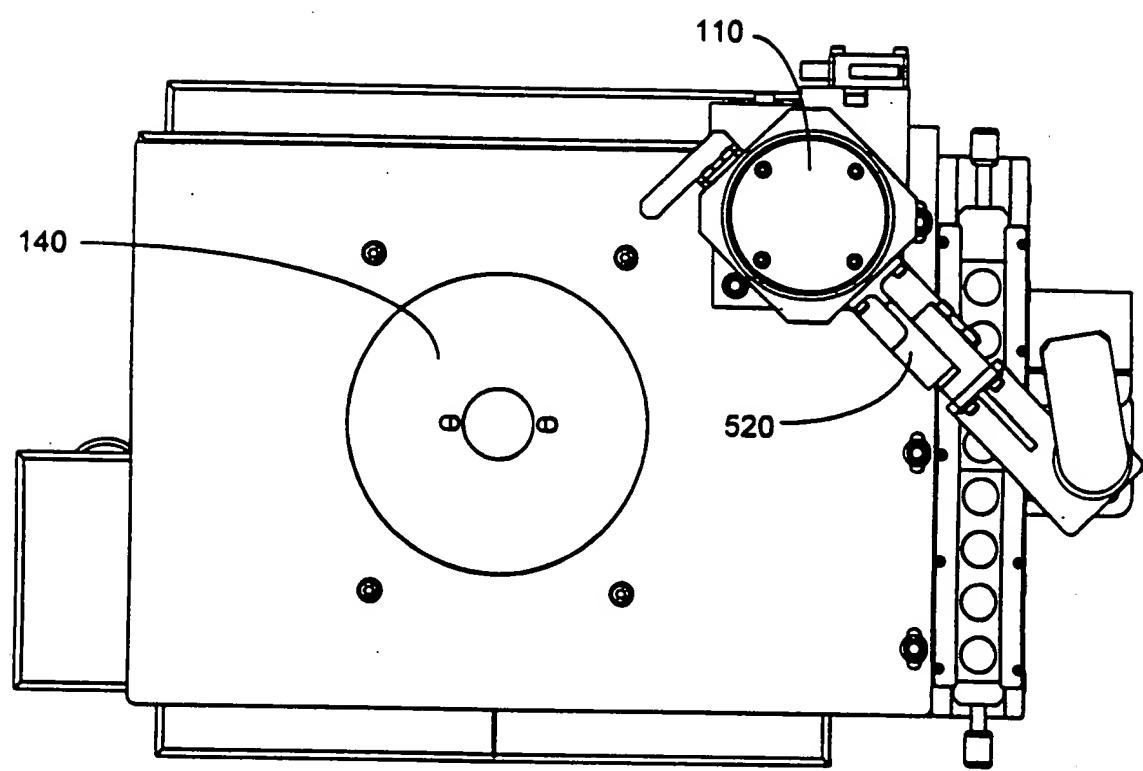


FIG. 11

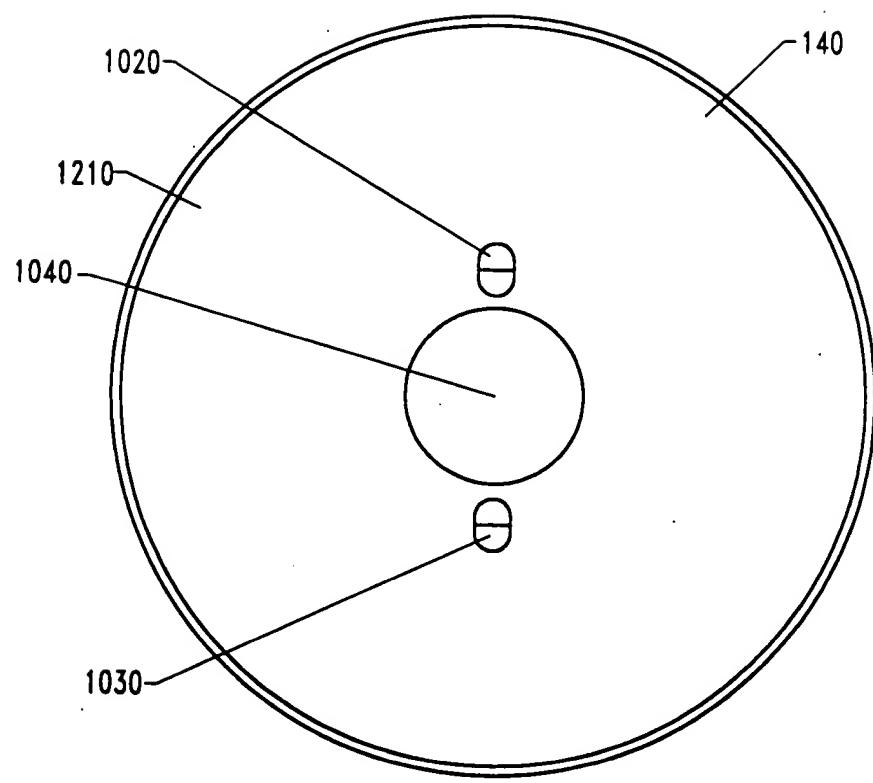


FIG. 12

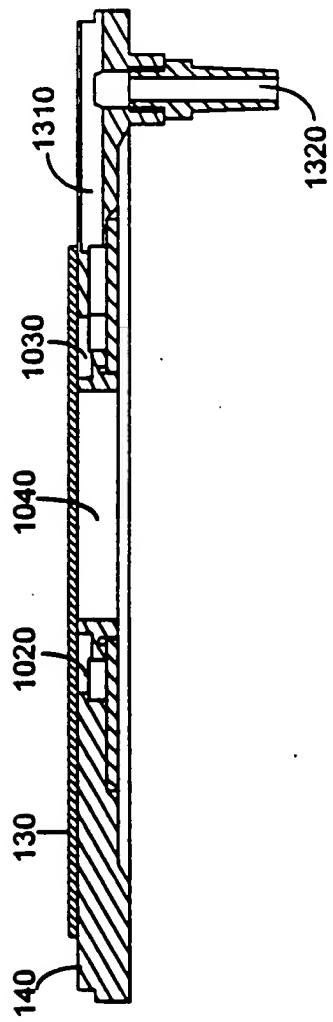


FIG. 13

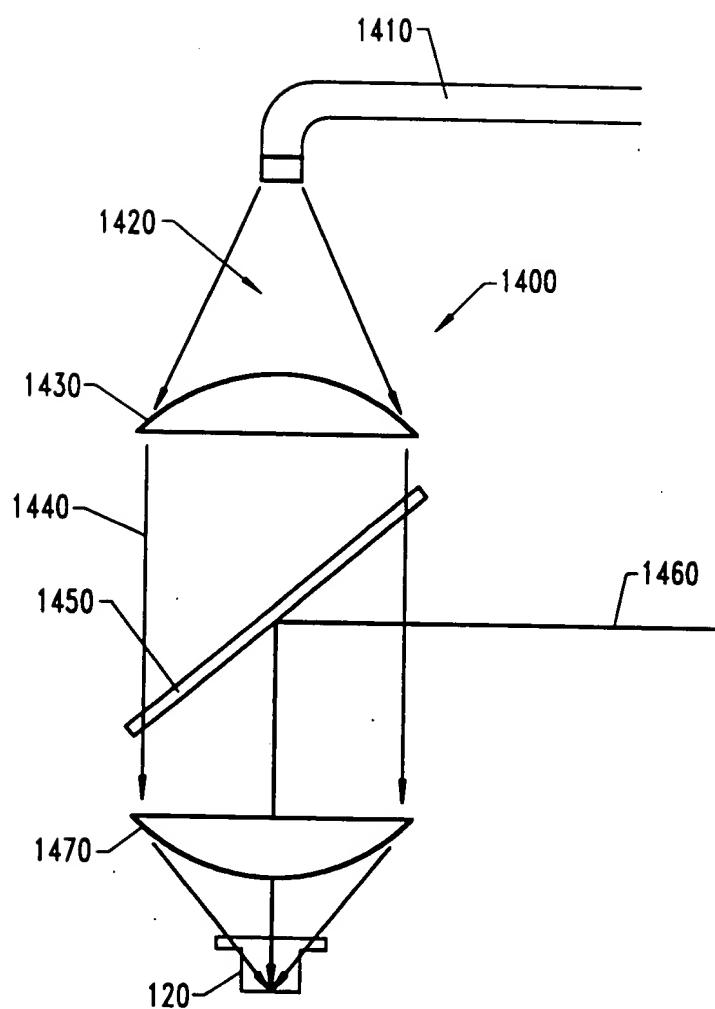


FIG. 14

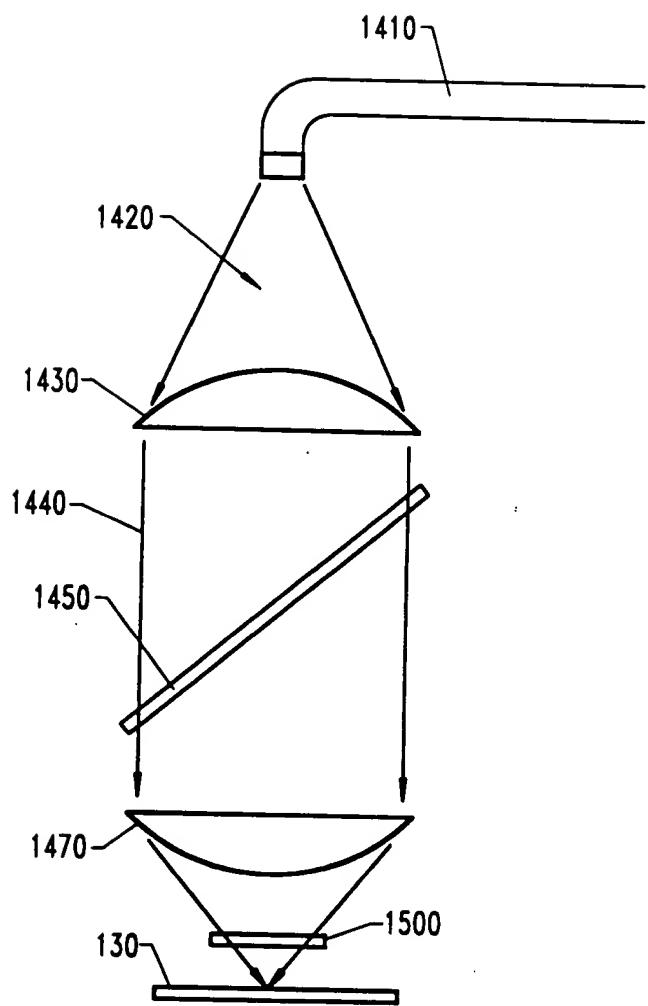


FIG. 15

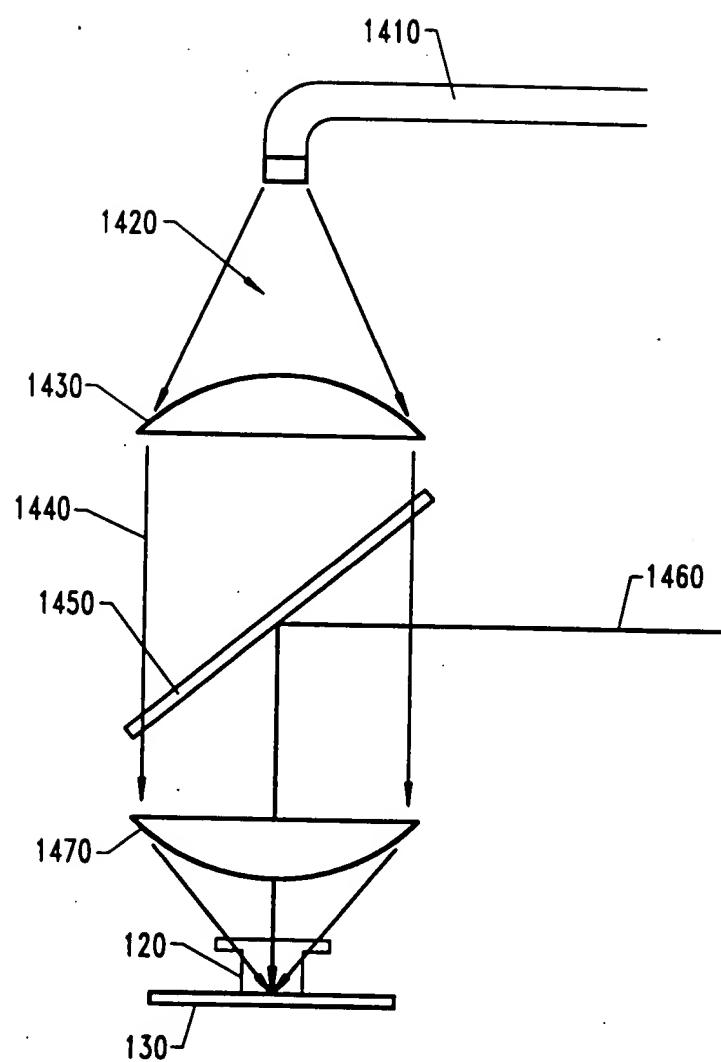


FIG. 16

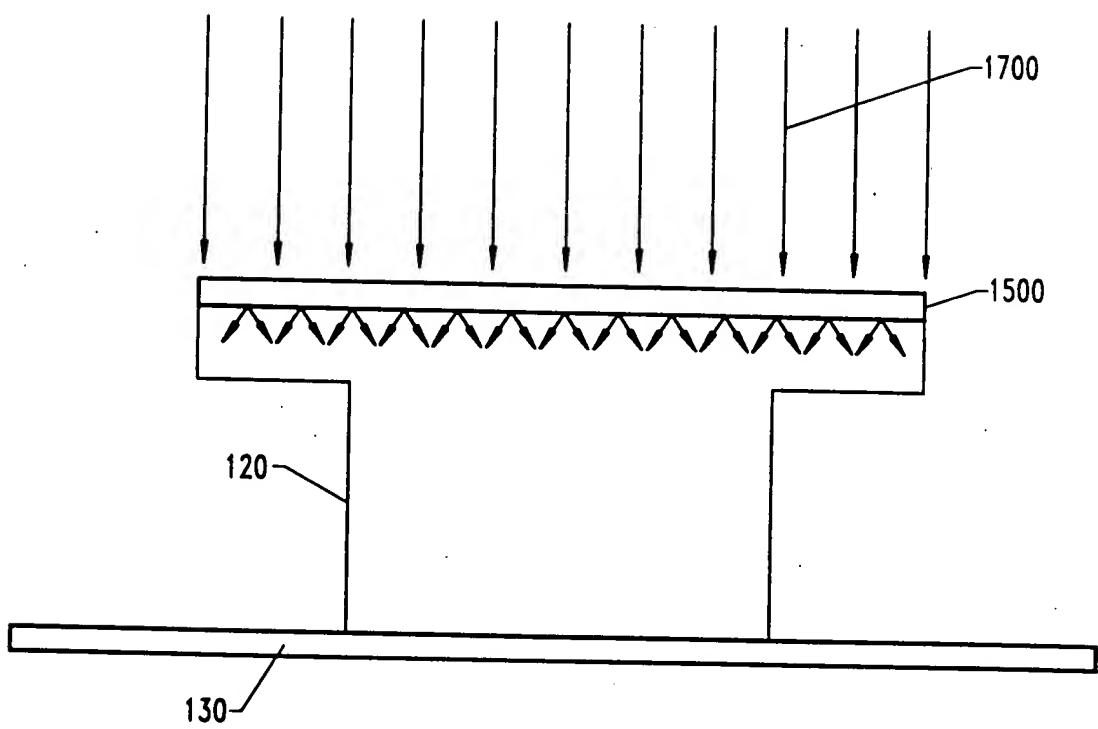


FIG. 17

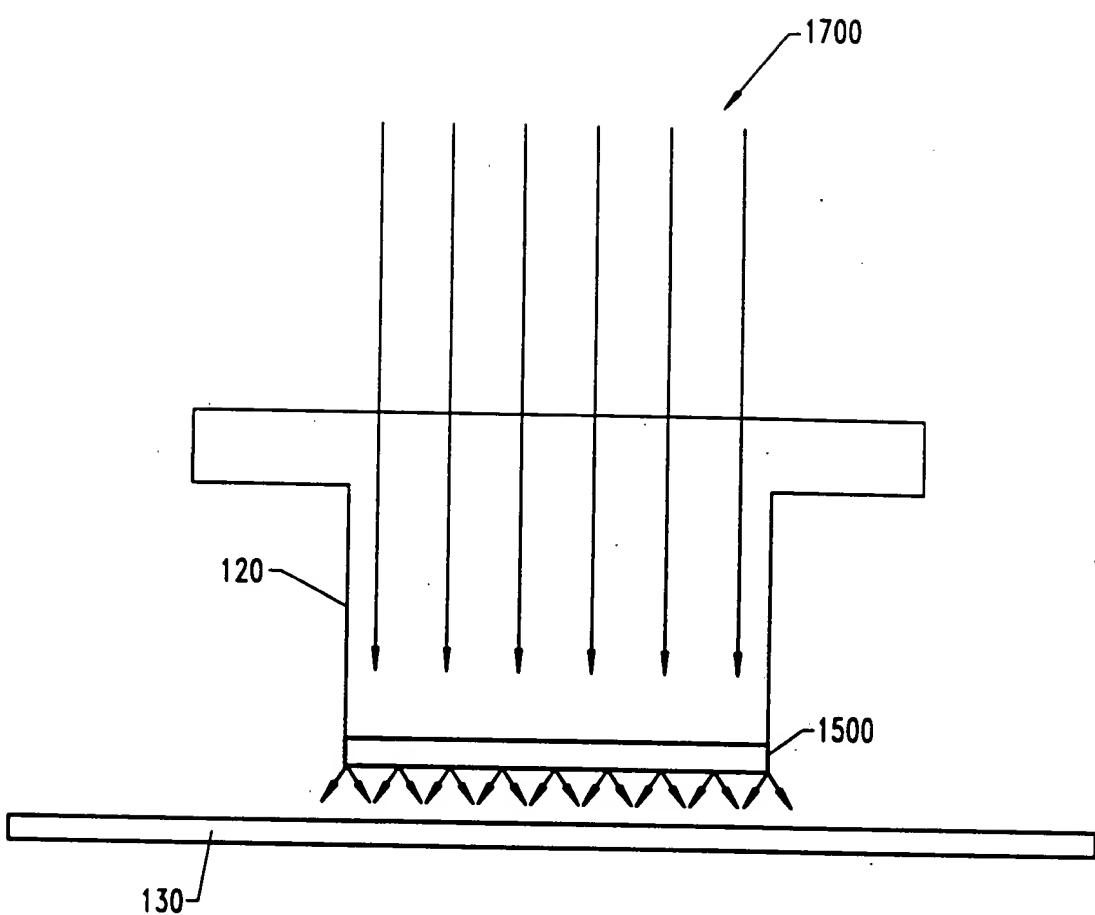
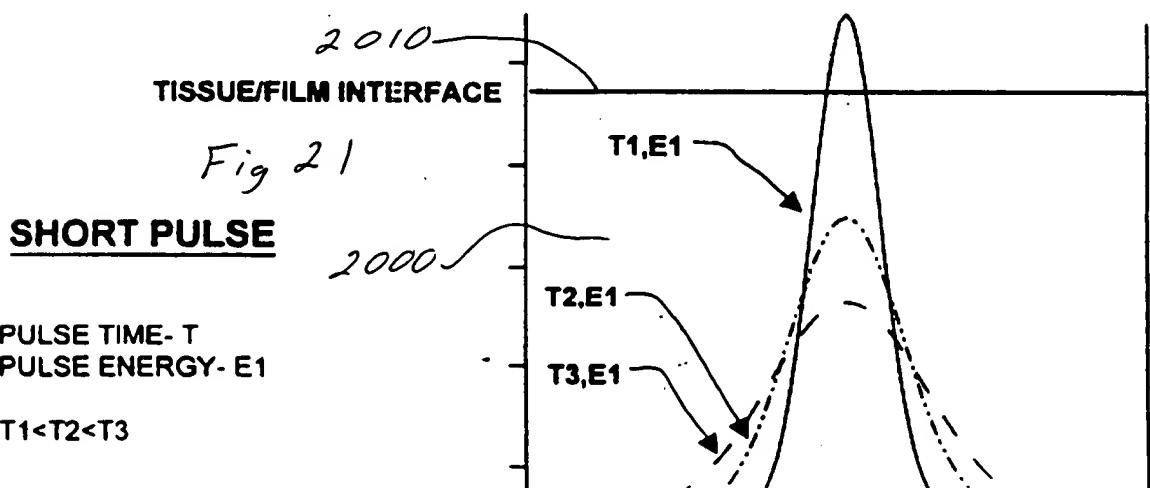
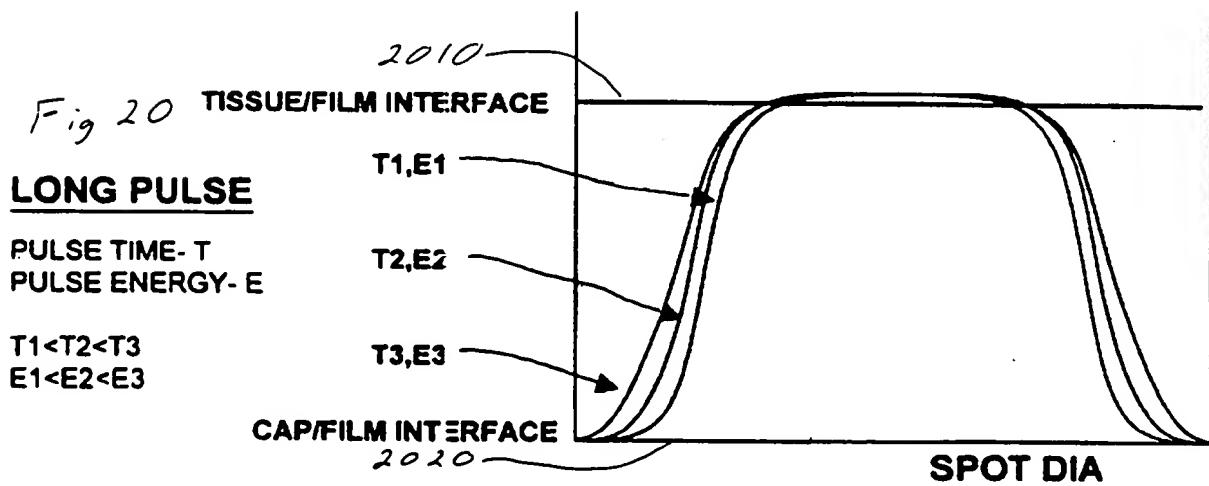
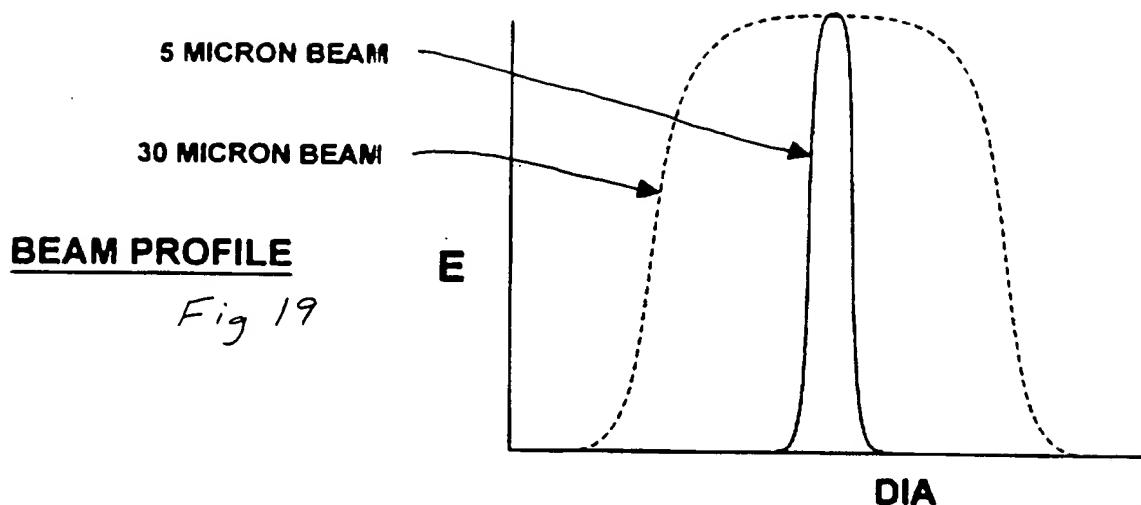
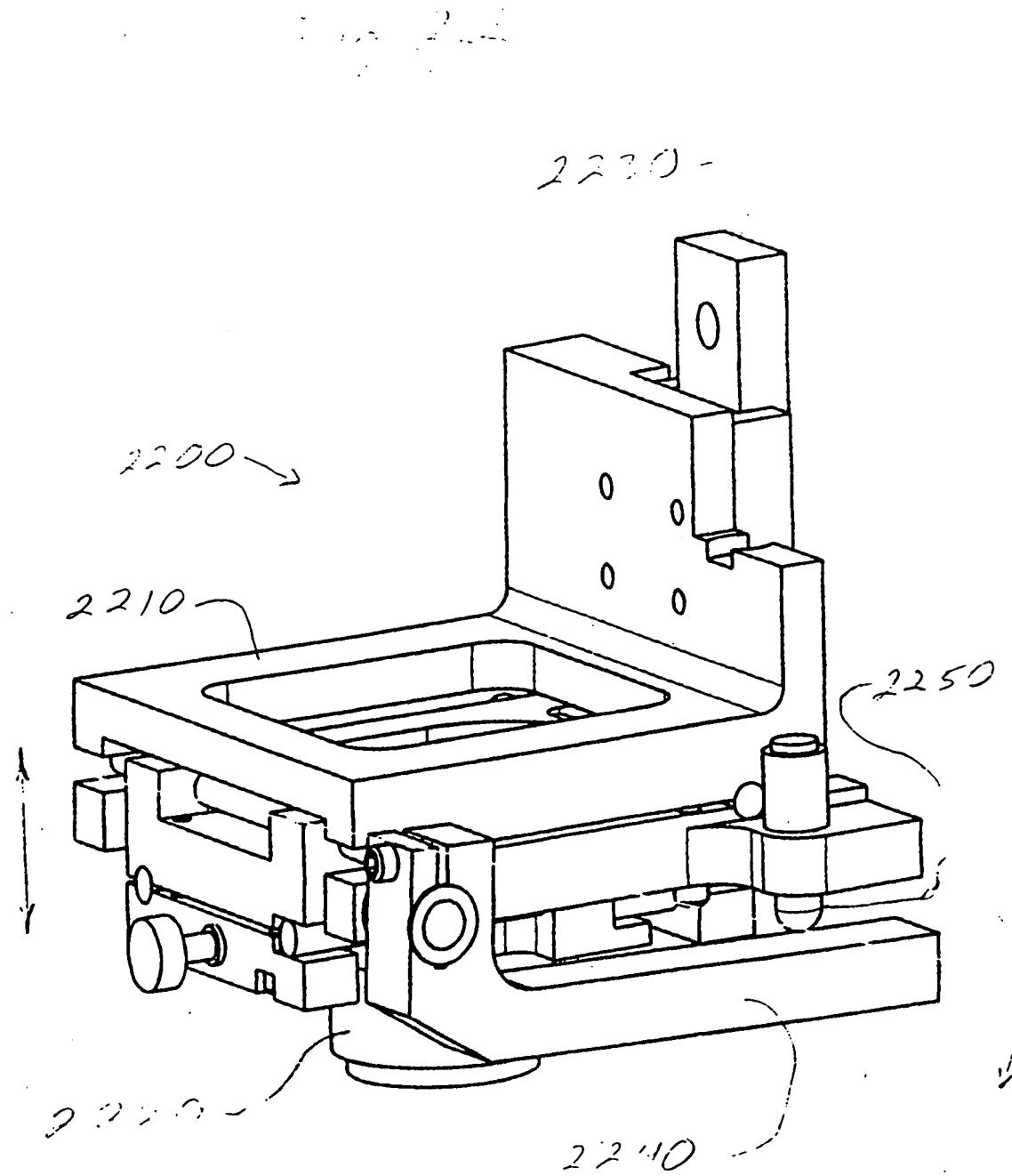


FIG. 18





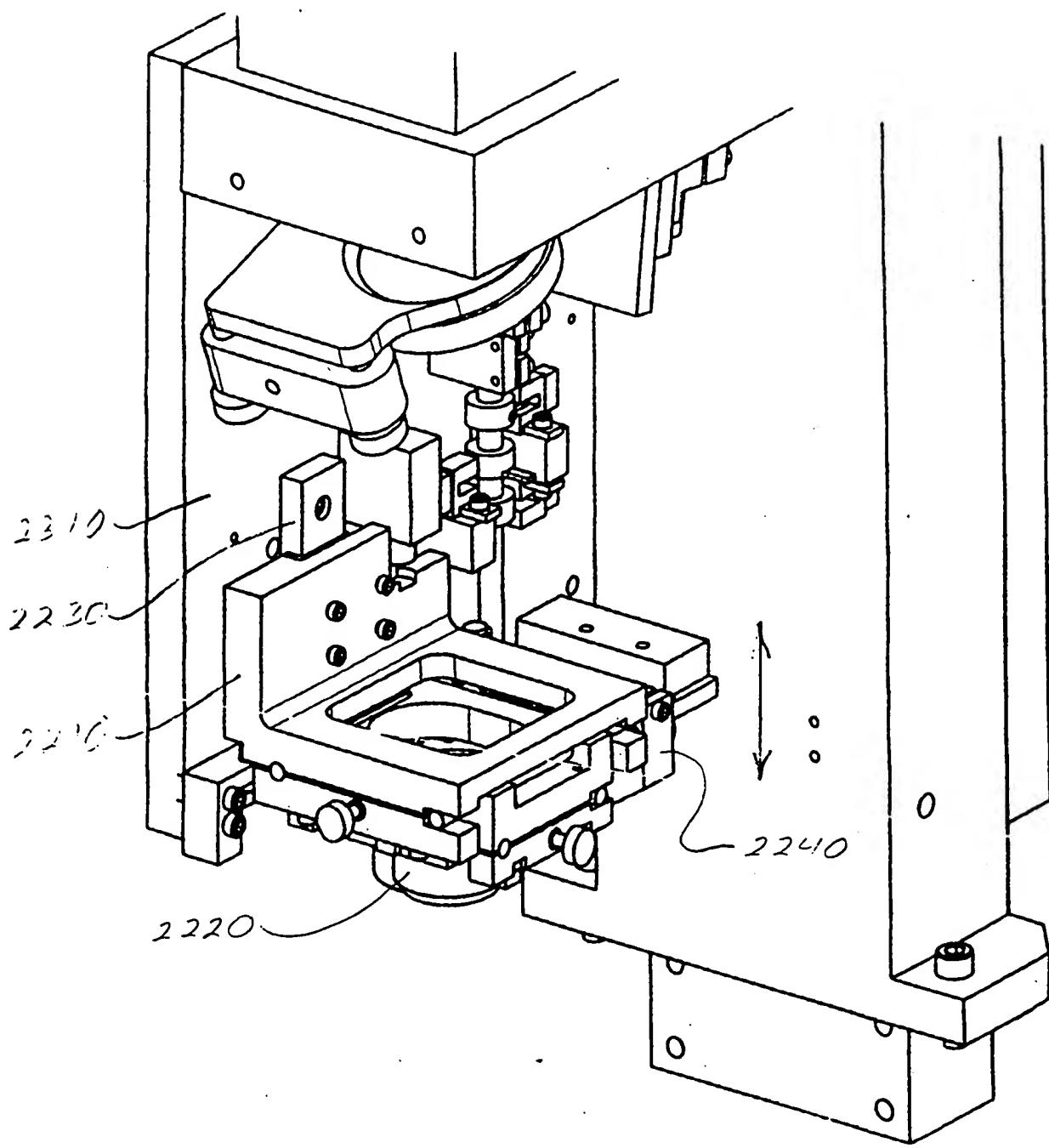


Fig 21

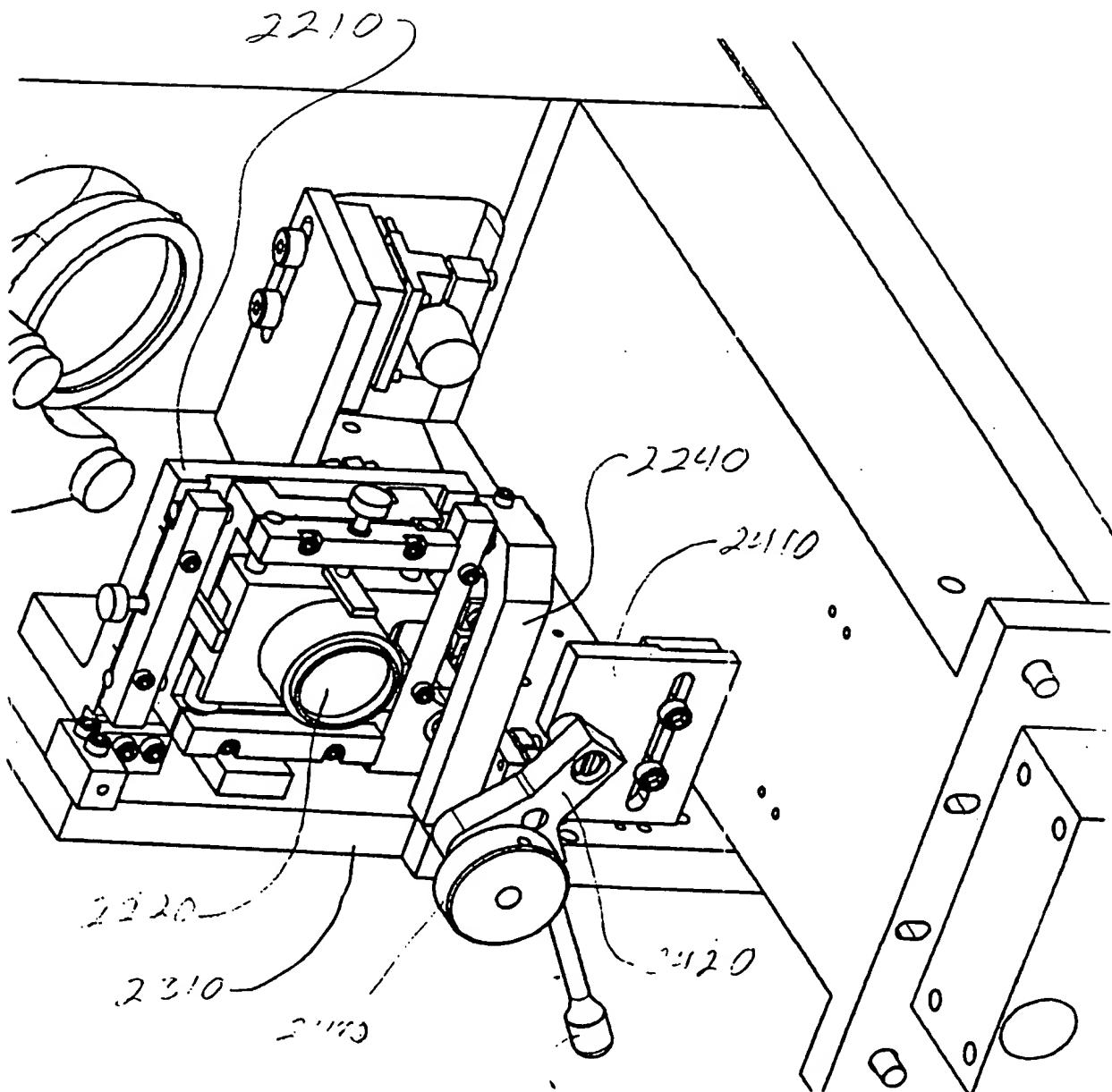
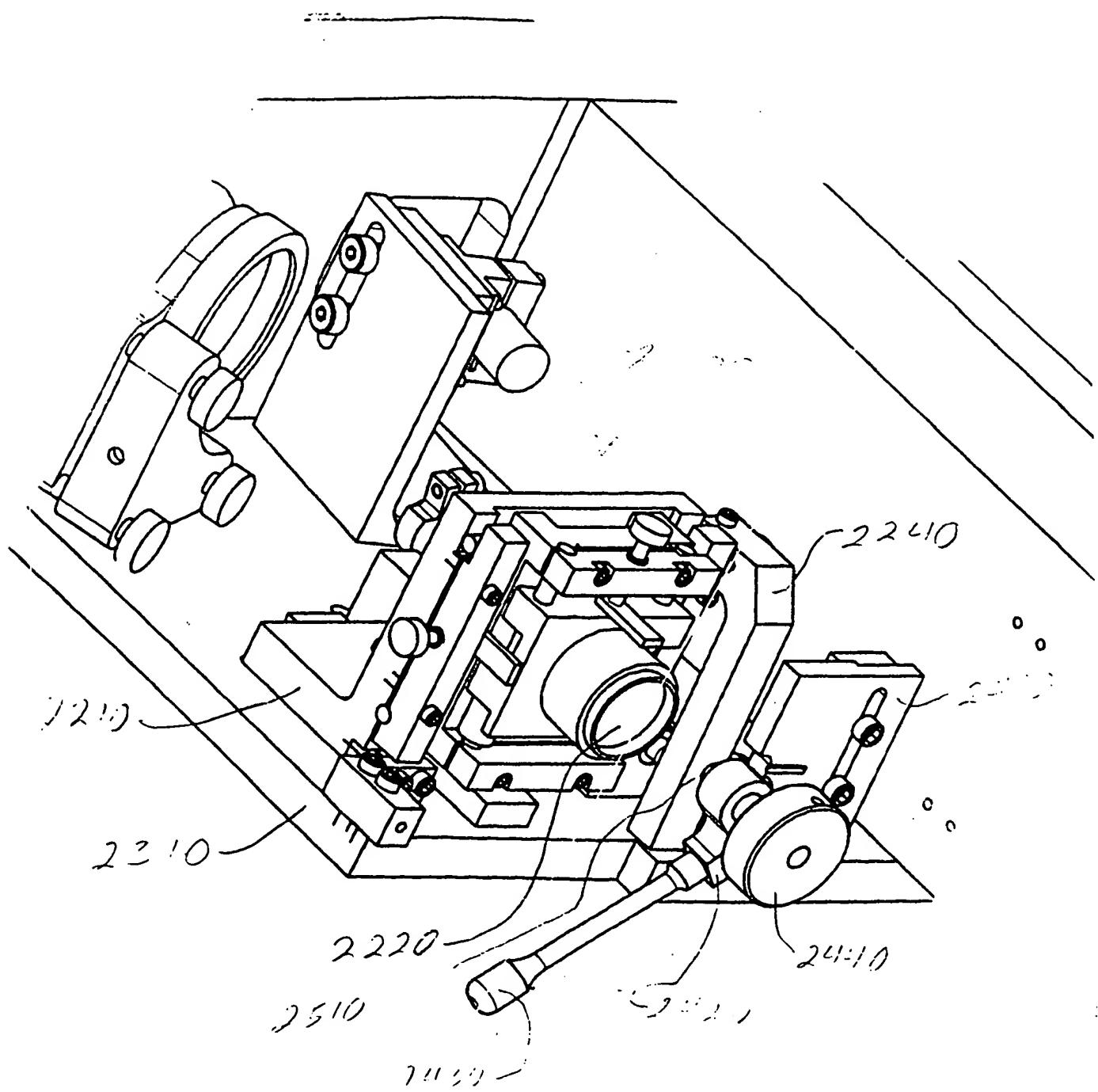


Fig 25



Inventors: Ann Bennett Lossing; Sherrie L. Ransom;
Steve Kunitake; Robert H. Reamey;
David F. Head; and Hala Al-Shawany
Attorney Docket No.: 17726-736
Client: Arcturus Engineering, Inc.

PROCESSING TECHNOLOGY FOR LCM SAMPLES

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part under 35 U.S.C. § 120 of copending U.S. Ser. No. 60/131,863, filed April 29, 1999, now pending, the entire contents of which are hereby incorporated herein by reference as if fully set forth herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates generally to the field of laser capture microdissection (LCM). More particularly, the invention relates to providing a biological sample with a polymer barrier prior to laser capture microdissection.

2. Discussion of the Related Art

LCM is a process by which cells and portions of biological tissue samples are acquired directly from tissue sections mounted on glass slides or other solid surfaces. The process involves placing a Capsure™ device, containing a thin-film polymer, onto the tissue section. Once the cells or tissue portions of interest (tissue targets) are located in the sample, a laser is focused over the tissue targets. When the laser is fired, the thin-film located directly above the tissue targets melts, flows down and adheres to the tissue targets. The Capsure™ device, holding the adhered tissue targets, is then removed from the tissue sample. The tissue targets are now stabilized on the Capsure™ device and ready for molecular analysis.

Currently, when Capsure™ devices make contact with a tissue section during LCM, the total working area of the Capsure™ device touches the surface of the tissue section. Due to the friable nature of tissue sections, loose material

(whole cell or macromolecular) is likely to adhere to the surface of the Capsure™ device during LCM. This is known as non-specific transfer. Since LCM sample recovery involves extraction of the material on the surface of a Capsure™ device, any non-specific material transferred during LCM can cause sample contamination and adversely affect the quality and accuracy of downstream analyses.

Heretofore, the requirement of reducing or eliminating non-specific transfer during LCM has not been fully met. What is needed is a solution that addresses this requirement. The invention is directed to meeting this requirement, among others.

SUMMARY OF THE INVENTION

The principal goal of the invention is to satisfy the above-discussed requirement of reduction or elimination of non-specific transfer during LCM. It was reasoned that by applying a protective barrier or coating to biological tissue sections prior to LCM, loosely adhered tissue would be retained beneath the barrier, whereas tissue targets adhered to the melted polymer would easily be removed with the Capsure™ device. Another goal of the invention is to improve visualization of the sample during LCM. Another goal of the invention is to stabilize and retard degradation of biological samples being used for LCM or other types of biological analysis. Another goal of the invention is to reduce contamination of biological samples during storage before or after LCM.

One embodiment of the invention is based on a method of processing a biological sample for laser capture microdissection, comprising: providing the biological sample; and applying a substance to the biological sample so as to provide a barrier between the biological sample and a surrounding environment. Another embodiment of the invention is based on an article of manufacture, comprising: a biological sample that is to undergo laser capture microdissection; and a barrier coupled to at least a portion of said biological sample. Another embodiment of the invention is based on an article of manufacture, comprising: a portion of a biological sample that has undergone laser capture

microdissection; and a barrier coupled to said portion of said biological sample. Another embodiment of the invention is based on a composition to process a biological sample for laser capture microdissection, comprising: a solvent; and solute in said solvent, said solute capable of forming a barrier on said biological sample. Another embodiment of the invention is based on a method, comprising: applying a substance to at least a portion of a biological sample that is to undergo laser capture microdissection.

Another embodiment of the invention is based on an apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising: a container adapted to provide a fluid source of said substance, said container including an orifice that defines a principal plane that is substantially parallel to a primary direction of movement that is to be taken by said biological sample while said substance is being applied. Another embodiment of the invention is based on an apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising: a dispensing device to deliver or apply a volume of fluid directly onto a biological sample. Delivery or application of the fluid volume to the sample can be in the form of a bead, droplet, spray dispersion, aerosol, spin-coat and/or drip-coat. Another embodiment of the invention is based on an apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising: a release layer; and a solid layer of said substance coupled to said release layer. Another embodiment of the invention is based on an apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising: a blade including an indexing surface and a surface to define a gap that defines a principal plane that is held at an acute angle with respect to a perpendicular to a primary direction of movement that is to be taken by said biological sample while said substance is being applied.

These, and other goals and embodiments of the invention will be better appreciated and understood when considered in conjunction with the following

description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference characters (if they occur in more than one view) designate the same parts. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

FIGS. 1A-1B illustrate micrographs of control samples that do not have a polymer barrier, appropriately labeled "prior art."

FIGS. 2A-2B illustrate micrographs of samples that do not have a polymer barrier imaged through a diffusing media, appropriately labeled "prior art."

FIGS. 3A-3B illustrate micrographs of samples with a polymer barrier, representing an embodiment of the invention.

FIGS. 4A-4B illustrate micrographs of CAPSURE devices having on them portions of samples that did not have a polymer barrier prior to acquisition by LCM, appropriately labeled "prior art."

FIGS. 5A-5C illustrate micrographs of CAPSURE devices having on them portions of samples that had a polymer barrier prior to acquisition by LCM, representing an embodiment of the invention.

FIGS. 6A-6B illustrate micrographs of samples that did not have a polymer barrier prior to acquisition by LCM, appropriately labeled "prior art."

FIG. 6C illustrates a micrograph a CAPSURE device having on it a portion of a sample that did not have a polymer barrier prior to acquisition by LCM, appropriately labeled "prior art."

FIGS. 7A-7B illustrate micrographs of samples and portion thereof that had a polymer barrier prior to acquisition by LCM, representing an embodiment of the invention.

FIG. 7C illustrates a micrograph of a CAPSURE device having on it a portion of a sample that had a polymer barrier prior to acquisition by LCM, representing an embodiment of the invention.

FIG. 8 compares fluorescence as a function of the number of tissue targets between non-coated samples and 5% 200W coated samples, representing an embodiment of the invention.

FIG. 9 illustrates a schematic perspective view of a method of processing a biological sample, representing an embodiment of the invention.

FIGS. 10A-10C illustrate schematic perspective views of a lamination process, representing an embodiment of the invention.

FIGS. 11A-11B illustrate schematic views of a coating process, representing an embodiment of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed in the following description of preferred embodiments. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

The purpose of this invention is to provide technology, that is compatible with LCM, to meet some or all of the following needs: prevent non-specific binding of sample to CapSure™; optimal visualization of samples; and stabilization of biological samples. The term "CapSure™" is used herein to generically refer to the combination of an integral portion of an analysis vessel

that includes an LCM transfer film and an LCM transfer film carrier. Of course, the invention is not limited to CapSure™.

The invention can include laminating or coating a biological sample (cells or a portion of tissue) mounted on a glass slide or other hard surface material, with a substance. Once the sample is coated, the substance acts to sequester the sample, providing a barrier between the sample and the surrounding environment. The purpose of this barrier is to prevent the sample from making contact with instruments or other items which may be used to manipulate the tissue.

The laminate or coating may be EVA (ethyl vinyl acetate) or some other polymer, modified to have specific properties required to facilitate this invention, or unmodified, and applied in a thickness to be determined. The laminate or coating will be applied to the sample by one of the following methods: dissolving the laminate or coating material supplied in powder, beads or other bulk form, in a solvent; dipping the slide containing the tissue sample into a quantity of the resulting solution; spraying or aerosolizing the laminate or coating solution onto the sample; delivering a volume of the laminate or coating solution onto the surface of the sample. Other application methods may include liquefying the laminate or coating and rolling the slide between rollers (the rollers may be heated and/or cooled); melting a thin sheet of laminate or coating onto the slide using a heat block; and melting a thin sheet of laminate or coating onto the slide with an IR (infrared) sweep. Additional application methods may include spin coating and/or doctor blading, optionally with the below discussed glider device. Also a laminate can be simply pressed onto the tissue without heat.

Application of the laminate or coating to the sample does not interfere with normal handling and processing of the sample, LCM or molecular analysis of the sample. The laminated or coated samples will have the same or better visual appearance as non-coated tissue samples. The laminate or coating will not impede or retard successful LCM transfer of cells or portion of the tissue or

prevent access to cells or portions of the tissue for any form of biological analysis.

The invention can include providing a clear coat of polymer on a tissue sample. One embodiment of the invention is based on ELVAXTM 200W diluted in 100% xylene. Xylene is a common histological solvent, known to be compatible with all tissue types and routinely used in tissue preparation for LCM. Rapid xylene evaporation expedites film formation (on evaporation, no polymerization, per se occurs) of the laminate or coating and drying of the sample. The invention can be applied directly to a slide after normal staining or processing. The invention therefore involves only one new step in sample preparation protocol.

The invention (some embodiments of which can be referred to as POLYSLIPTM and/or POLY-SLIPTM) was developed as a means of coating the tissue section in order to isolate the surface of the section from the surface of the CapsureTM device. This method of contact surface separation would eliminate the possibility of non-specific material transferring from tissue to CapsureTM devices. Consequently, only tissue targeted for microdissection would be transferred to the CapsureTM device after LCM. This ensures the homogeneity of cell samples collected by LCM and prevents contamination of LCM samples with unwanted macromolecules. During our preliminary testing of the invention, both coated and non-coated tissue sections were microdissected. Pictures of CapsureTM devices used in both procedures are shown in FIGS. 4A-4B, 5A-5C, 6C and 7C. Referring to FIGS. 4A-4B, 5A-5C, 6C and 7C, it is very evident that non-specific material is not present on CapsureTM devices used in laser capture microdissection of inventive coated tissue sections.

The invention can be made from an ethylene vinyl acetate (EVA) polymer, ELVAXTM 200W, diluted with 100% xylene. ELVAXTM 200W is available in pellet or powdered form, which facilitates dissolution in xylene. Since the xylene evaporates rapidly, the invention quickly forms a film and dries on the sample. The invention can be applied directly to tissue sections

after staining and processing, as a single additional step in the sample preparation protocol.

We wish to evaluate the feasibility of developing the invention as a product. Our initial feasibility testing program is outlined below.

Initial Product Specifications

1) Performance

- a) Reduction of non-specific transfer by 100% when compared to non-coated tissue sections
- b) Target tissue transfer and recovery efficiency of 100% when compared to transfer and recovery from non-coated sections
- c) Retention of current tissue visualization ability and target capture features (diameter of wetting area; collateral (immediately peripheral to target area) material transfer), relative to non-coated tissue
- d) Demonstration of compatibility with currently used molecular processing methods (PCR, GelElectrophoresis, Molecular Hybridization methods...)
- e) Demonstration of stability of the invention for at least 90 days
 - i) in xylene solution, sealed in a glass container, at room temperature
- f) Demonstration of stability of tissue sections coated in the invention for at least 90 days
 - i) no evidence of tissue degradation, or reduction in macromolecular content or function
 - ii) no change in LCM transfer efficiency during that time

2) Application Method

- a) Demonstration tissue sample types
 - i) 5 micron paraffin-embedded tissue sections
 - ii) Leukocyte enriched blood smears
- b) Application process
 - i) Apply even coating

- ii) Air dry 5 minutes
 - iii) Perform LCM according to standard procedures
 - c) Expected application time - 5 minutes (including drying)
 - d) Special application equipment requirements - undetermined
 - e) Additional LCM instrumentation or equipment modification requirements - none
- 3) Formulation and Packaging
- a) small volume (25-50 ml.) of fluid in sealed glass container
- Technical Feasibility Evaluation Goals
- 1) Product Design Specifications
 - a) Define preliminary formulation
 - b) Define preliminary application method
 - 2) Theoretical Performance Report
 - a) LCM transfer efficiency (compared to un-coated samples)
 - b) Macromolecule (DNA, RNA, Protein) extraction and recovery efficiency (compared to un-coated samples)
 - c) Compatibility with current molecular analysis methods (PCR)
 - d) Stability of preliminary formulation
 - e) Stability of tissue sections coated with the invention (compared to un-coated samples)

Prototypes were made with EVA (i.e., ELVAXTM 200W) dissolved in xylene (i.e., 100% xylene) with and without dye. Operational results with these prototypes are discussed below.

Referring to FIGS. 1A-1B, 2A-2B, and 3A-3B, the visualization advantages of the invention can be appreciated. FIGS. 1A-1B show two views of a sample without a polymer barrier. FIGS. 2A-2B show two views of the sample shown in FIGS. 1A-1B, but imaged through a diffusing media. FIGS. 3A-3B show two views of a sample with a polymer barrier. It can be appreciated that the quality of the visualization shown in FIGS. 3A-3B is surprisingly superior compared to the visualization shown in either FIGS. 1A-1B or FIGS. 2A-2B.

Referring to FIGS. 4A-4B and 5A-5C, the non-specific transfer advantages of the invention can be appreciated. FIGS. 4A-4B show images of an LCM transfer film (seen through an LCM transfer film carrier) both before LCM acquisition of a sample portion from a sample that did not have a polymer barrier (FIG. 4A) and after (FIG. 4B). FIGS. 5A-5B show an images of an LCM transfer film (seen through an LCM transfer film carrier) both before LCM acquisition of a sample portion from a sample that had a polymer barrier (FIG. 5A) and after (FIG. 5B). FIG. 5C shows a close-up of the sample portion shown in FIG. 5B. The substantial absence of non-specific transfer with the use of the invention is clearly evident.

Referring to FIGS. 6A-6C and 7A-7C, both the visualization and non-specific transfer advantages of the invention can be appreciated. FIGS. 6A-6C show visualizing before LCM, visualizing after LCM, and the resulting transferred portion of a sample that did not have a barrier, respectively. In contrast, FIGS. 7A-7C show visualizing before LCM, visualizing after LCM, and the resulting transferred portion of a sample that had a barrier, respectively. It can be appreciated that visualizing is significantly improved and non-specific transfer is significantly reduced as a result of the barrier. Currently, when Capsure™ devices make contact with a tissue section during Laser Capture Microdissection (LCM), the total working area of the Capsure™ device touches the surface of the tissue section. Due to the friable nature of tissue sections, loose material (whole cell or macromolecular) is likely to adhere to the Capsure™ device during LCM. This is known as non-specific transfer. Since LCM sample recovery involves extraction of the material on the surface of a Capsure™ device, any non-specific material present can cause sample contamination.

The invention was developed as a means of coating the tissue section in order to isolate the surface of the section from the surface of the Capsure™ device. This method of contact surface separation would eliminate the possibility of non-specific material transferring from tissue to Capsure™ devices. Consequently, only tissue targeted for microdissection would be

transferred to the Capsure™ device after LCM. This ensures the homogeneity of cell samples collected by LCM and prevents contamination of LCM samples with unwanted macromolecules.

Applying the substance to a tissue section prior to LCM merely requires applicators and application methods that are compatible with current tissue preparation methods. Extracting substance coated material from the Capsure™ device surface is compatible with extraction methods, and buffers, and can be used under the various temperature requirements normally part of molecular analysis protocols.

Substance Formulations Tested

Table 1 lists some formulations that have been used in different experiments to demonstrate their ability to abate non-specific transfer and still permit microdissection of tissue sections.

12\38

Table 1
Compositions

POLYMER	SOLVENT	%	NS detected on sample
Elvax 200W	Cyclohexane	.9	None
Elvax 200W	Cyclohexane/Ether 30/70	.2	Low
Elvax 200W	Cyclohexane/Ether 30/70	.6	None
Elvax 200W	Cyclohexane/Ether 30/70	.9	None
Elvax 200W	Cyclohexane/Pentane 30/70	.9	None
Elvax 200W	Xylene	.9	None
Elvax 200W	Xylene	1	None
Elvax 200W	Xylene	2.5	Low
Elvax 200W	Xylene	5	None
Elvax 200W	Xylene	7.5	None
Elvax 200W	Xylene	10	None
Elvax 450	Cyclohexane	1	None
Elvax 450	Xylene	1	None
Paraffin Mp. 56°C	Xylene	1	Low
Paraplast™	Cyclohexane	1	Low
Poly α -methyl styrene	Xylene	1	None
Polyester Wax Mp. 37°C	Xylene	1	Low

The following section is descriptive generally for either liquid or solid embodiments of the invention. In most cases a polymeric material is desirable, but in some cases the materials are not polymers. For example, paraffin and polyester waxes are useful, but do not fall into the class of materials commonly referred to as polymers.

In addition to using a solid film and coating from solution, one could use a system which undergoes curing. The curing could be from monomers, or could involve crosslinking of a polymeric solution. If the curing is done from monomers, it would be possible to have a solvent free liquid system. Examples of curable systems can be acrylic, urethane, or epoxy chemistry.

The materials for use in the invention must be optically transparent, and should have little to no color. In order to use them in the liquid application method, they must be soluble or dispersible in the solvent used. It is desirable that they be film-forming materials, but that is not an absolute requirement. The film of material must have a cohesive strength which is less than the tissue so that only the tissue in contact with the selected tissue portion is transferred. In some cases it is useful for the inventive materials to undergo softening when heated.

The following materials are suitable for use with the invention:

Waxes or low molecular weight oligomers – Paraffin, Polyester, Wax

Polyethylene – especially low molecular weight

Ethylene co-polymers such as poly (ethylene-co-vinyl acetate) (EVA), and poly(ethylene-co-acrylic acid) (EAA).

Acrylates, urethanes, epoxies

Water borne polymers and oligomers – such as Polyvinyl alcohol (PVA), Polyvinyl Pyrrolidone (PVP), Polyethylene oxide (PEO), Polyethylene glycol (PEG), and Poly acrylic acid.

Polyisobutylene

Methods of Application

1) Referring to FIG. 9, an apparatus for applying the inventive composition to a glass slide mounted biological sample is depicted. A slide 200 is pulled along underneath an orifice 210 in a container 220 through which a barrier forming solution 230 flows.

2) Referring to FIGS. 11A-11B, one method of application which is a subset of the invention is a device 350 which fits over a microscope slide 300, indexes off of a surface 310, and creates a fixed gap which spreads the fluid evenly when pulled across the surface of the slide. The device 350 includes a first recess 320 that indexes on the surface 310. The device 350 also includes a second recess 330 that sets a gap for spreading the coating 320. An operator can slide the device 350 across the slide 300 to form an even coat of substance. This device can be referred to as a glider.

An experiment will now be described. The objective of this experiment was to test the level of Non-Specific abatement provided by the inventive formulation 5% ELVAXTM 200W in xylene (5% 200W).

Two Hematoxylin/Eosin(H&E)-stained brain tissue sections from the same lot were selected for this experiment. One was coated with a thin layer of 5% 200W. An uncoated H&E-stained brain section was used as a control. Target cells (500, 250, 100, 50, 10 and 5 per section) were Laser Capture Microdissected (LCM) from each section. One blank CapsureTM device was included as a control for each sample set.

Cellular material was extracted using a Proteinase K - based extraction protocol. Extracted material was treated with PicoGreen[®] fluorescent DNA Quantitation solution and then quantitated on a Packard Fluorcount[®] Fluorimeter.

Total fluorescence for samples and controls was measured on the Fluorcount[®] Fluorimeter and recorded. Background fluorescence in the system was established by measuring the total fluorescence of a volume of the Proteinase K extraction buffer equivalent to that of each sample and control.

As shown in Table 2 and FIG. 8, non-coated tissue samples gave variable fluorescence readings, with no apparent correlation between the number of cells in the sample and sample fluorescence. On the other hand, 5% 200W coated tissue samples showed a reasonably linear relationship ($r^2 = 0.9751$) between the number of targets and both total and corrected sample fluorescence. Coated samples also had an overall lower fluorescence level than non-coated samples.

It is reasonable to conclude that the presence of non-specific material could account for both the variability of fluorescence in the non-coated tissue samples, as well as the increased fluorescence, when compared to coated tissue samples.

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Table 2

Sample Fluorescence

Coated vs. Uncoated Samples

	TARGETS	500	250	100	50	10	5	3 Blank
Non- Coated Tissue	TSF	44531	18437	26166	27125	19882	6657	3852
	CSF	42645	16551	24281	25238	17106	4770	1965
5% 200W Coated Tissue	TSF	25866	18507	9276	7349	4010	4478	3680
	CSF	23980	16621	7389	5463	2124	2592	1794

LEGEND -- TSF - Total Sample Fluorescence; CSF - Corrected Sample Fluorescence (Digestion Buffer Blank
TF was subtracted from Sample TF)

Another experiment will now be described. The objective of this experiment was to test the level of non-specific abatement provided by the inventive composition of ELVAXTM 200W in xylene (5% 200W).

Unstained human brain sections were used for this experiment. Sections were coated with 5% 200W. Control sections were uncoated. Laser capture microdissection (LCM) was done in highly nucleated areas of the section, with each target acquiring between 1 and 2 nuclei per shot, as well as matrix material. 50 LCM targets were captured in each sample and 10 samples were taken from both the coated and non-coated sections.

Samples were extracted with a Proteinase K extraction buffer. The extracted samples were treated with PICOGREENTM Fluorescent DNA Quantitation solution and then quantitated on a Molecular Dynamics FLUORIMAGERTM.

Fluorescent readings were taken for each sample and the median pixel fluorescence (MPF) for each sample was recorded. Background fluorescence in the system was established by measuring the total fluorescence of a volume of the Proteinase K extraction buffer equivalent to that of each sample and control. A corrected MPF (cMPF) was determined by subtracting the background fluorescence from the MPF of each sample and control.

The fluorescence data is shown in Table 3. The average cMPF value for 10 samples from uncoated sections was 15.75 (s.d. 9.58). The average mPF value for 10 5% 200W samples was 3.30 (s.d. 0.67). The high variability of sample fluorescence in the uncoated sections, as compared to coated sections, would seem to indicate the presence of non-specific material in the sample.

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Table 3

Fluorescent Readings

Sample #	Uncoated Section Control cMPF	5% 200W Coated Section Sample cMPF
1	6.46	3.54
2	4.16	2.87
3	27.52	3.34
4	8.50	3.42
5	7.65	3.54
6	12.46	3.30
7	19.31	3.06
8	15.46	2.91
9	31.57	4.84
10	24.45	2.22
Mean	15.75 ± 9.59	3.30 ± 0.67

An experiment will now be described. The following materials were used:

slides with blood-smear tissue samples;
blank slides with no tissue sample for polymer coating only;
polymer solutions: paraffin wax with cyclohexane solvent;
Slide glider of clearance of 0.015" and width 25.4", and slide spinner with slide holder, both were used for spreading polymer solutions.

Regular CaptureTM devices and 4 um rail CaptureTM devices were experimented with. Life savers are adhesive acrylic "doughnuts" which adhere to the bottom of the CaptureTM device and define an extraction volume of 10 microliters (well diameter = 0.125 in...). The objective of this experiment was to test the level of Non-Specific abatement provided by the inventive formulations described in the next paragraph.

Two sets of polymer solutions with cyclohexane solvent at concentration of 2.5% (g wt. polymer by ml volume solvent), and the other set included each polymer mixed with Cyclohexane at 5% concentration. 2.5% solutions were prepared by mixing 0.25g of polymer with 10 ml of solvent, and similarly 5% solutions were made by mixing 0.50 g of polymer with 10 ml of solvent. Polymers normally dissolved after placing bottled solutions in water bath at 32.5 °C for 10-15 minutes. Four solutions in all were made based on two polymers and two concentrations.

Coated each of the polymer solutions at 2.5% concentration on three blood tissue slides, using the glider method. When applying with glider, 30 uL volume of polymer solution was pipetted just in front of the tissue and immediately spread with the glider held at 30° angle with respect to the perpendicular and drawn quickly over the slide. Also coated each of the 5% polymer solutions on one blood tissue slide, using the slide spinner. When using spinner method, 40 uL volume of polymer solution was placed in the slide holder so that 20 uL went in each hole at top of holder. Each slide was spun at high speed and time settings (DD settings).

Eight slides in all were coated, 6 using each of the 2.5% polymer solution applied with the glider (3 slides with Paraffin and 3 with Polyester-Wax), and 2 using each of the 5% polymer solution applied with the spinner (1 with Paraffin and 1 with Polyester-Wax). The similar coated slides were used for different Capsure™ device types (regular and rail) and different extraction methods (with or no life savers). Please see experiment outline for clarification.

Applied each of the 2.5% and the 5% polymer solutions on a blank slides (4 slides all together) with no tissue sample on slide. This is done to test for any fluorescence contribution from the polymer coating.

After polymer solution application, every slide was left under the hood for 45 min to dry then placed in desiccator for 4 hours before performing LCM. The thickness of the polymer layer is estimated at 4-6 um or less.

For each polymer coated slide, 4 Capsure™ devices were used. For sets using regular Capsure™ devices and no life saver extraction method, LCM was performed at 5 locations, one in the middle and the others around, half way between middle point and edge. In each location, 15 shots were fired, totaling to 75 shots per Capsure™ device. For sets using rail Capsure™ devices and regular Capsure™ devices with life saver extraction method, 75 shots were fired at center of Capsure™ device. Acquisition was performed using a commercial LCM instrument at setting of 0.8-1.0 ms duration and 50-80 mW power, with multiple firing (2-3 times). An LCM instrument was used to confirm the number of successful shots and verify extraction in post stained Capsure™ devices.

Extraction was performed on the paraffin coated blank slides and paraffin coated blood smear slides, using regular and rail Capsure™ devices, with or without life savers. These samples had reasonable shot capture efficiency and low non specific cell count . At a later time, PCR was performed on 2.5% Paraffin coated blood smear samples. PCR was successful.

The results summarized in Table 4 below show good tissue transfer, low levels of non-specific cells, and effective extraction of DNA from cells.

Blank paraffin coated slides (2.5 and 5%) were also extracted, and pico green analysis and visual inspection indicated no fluorescence contribution from polymer.

To verify extraction, the Capsure™ devices are restained and examined with an LCM instrument to visually determine extraction efficiency. Apparently most Capsure™ devices were left in heating block during extraction too long which resulted in melting of Capsure™ devices' surfaces. Only five Capsure™ devices were not damaged and verification was performed on them, showing that extraction occurred 100%.

Table 4

LCM Results for Blank and Blood Smear Slides Coated with Paraffin in
Cyclohexane Solutions

Sample	Cap #	Shots attempted	Shots capture eff.%	Estimated cells captured (visual)	Estimated Non-specific cells (visual)	Extraction eff.% (visual)
Blank (no tissue) with 2.5% soln Paraffin with glider	1B*	75			0	(not performed)
	2B	75			0	
	3B	75			0	
	4B	75			0	
	total	300			0	
2.5% Paraffin with glider regular caps no life saver	1B	75	45%	42	4	***
	2B	75	92%	89	100	100%
	3B	75	80%	76	0	***
	4B	75	92%	96	6	100%
	total	300	78%	303	110	*****
2.5% Paraffin with glider regular caps with life saver	1B	75	11%	8	0	***
	2B	75	89%	79	1	***
	3B	75	92%	76	0	***
	4B	75	97%	92	0	***
	total	300	72%	255	1	
2.5% Paraffin with glider rail caps with life saver	1R*	75	95%	79	0	***
	2R	75	89%	93	0	100%
	3R	75	100%	87	0	***
	4R	75	97%	100	0	100%
	total	300	95%	359	0	
5% Paraffin with spinner regular caps no life saver	1B	75	63%	52	6	***
	2B	75	88%	74	22	100%
	3B	75	81%	78	11	***
	4B	75	79%	69	11	***
	total	300	78%	273	50	***

* B = caps manufactured with automated method, and R = rail caps of 4 um height

*** These caps' surfaces were deformed due to staying in heat block too long during extraction

Another example will now be described. The following materials were used:

Tissue Samples: Nine blood-smear tissue samples were used, one for control (no invention), and eight for inventive polymer coating.

Polymer Solutions: Paraffin and Polyester Wax polymers were used along with Cyclohexane and xylene solvents.

Slide glider of clearance of 0.015" and slide spinner, both were used for spreading polymer solution.

CaptureTM devices made from new manufacturing method (type B) were used. CaptureTM devices came from lots #99K15B and #99L16B.

Two sets of polymer solutions were prepared, one set included each polymer mentioned above mixed with Cyclohexane, and the other set included each polymer mixed with xylene solvent. Each polymer solution was prepared by mixing 0.5 g polymer to 20 ml of solvent (2.5% wt. by volume solutions). Polymers normally dissolved after placing bottled solutions in water bath at 32.5 °C for 10-15 minutes. Four solutions in all were made based on two polymers and two solvents.

Coated each of the polymer solutions on two blood tissue slides, using the glider method on one slide and the spinner method on the other. For the glider method, 30 uL volume of polymer solution was pipetted just in front of the tissue and immediately spread with the 0.015" glider held at 30° angle with respect to the perpendicular. For the spinner method, 40 uL volume of polymer solution was placed in the slide holder so that 20 uL went in each hole at top of holder. Each slide was spun at medium speed and time (cc settings). Eight slides in all were coated, 4 using each of the polymer solution applied with the glider, and 4 using each of the polymer solution applied with the spinner.

After polymer solution application, every slide was left under the hood for 4 hours to dry then placed in desiccator overnight before performing LCM. The thickness of the polymer layer is estimated at 4-6 um or less.

For the control and each polymer coated slide, 4 CaptureTM devices were used. For each CaptureTM device LCM was performed at 5 locations, one

in the middle and the others around, half way between middle point and edge. In each location, 15 shots were fired (except for control's two first Capture™ devices at 10 shots/location) at LCM instrument setting of 1-1.2 ms duration and 52-65 mW power. Each Capture™ device then should have 75 shots total attempted. Fluorescence light was not available when firing shots. However, an LCM with fluorescence Capture™ deviceability was used to confirm number of successful shots afterwards.

Extraction was performed on the control and only half of the coated tissue samples due to low shot capture efficiency and/or high non-specific count on some sample Capture™ devices. Extraction was not performed on the later samples.

The results of this example are summarized in Tables 5 and 6. Capturing efficiency was high for control samples (no inventive coating) as well as Paraffin in Cyclohexane ones (using glider) as indicated in Table 4 Non-specific count was high in control Capture™ devices (100-500) while it dropped dramatically for samples mentioned above (0-131).

Extraction on control and selected inventive samples was successful 100% based on visual inspection (restaining Capture™ devices post extraction). Quantification of cells captured, however, is difficult to estimate from the pico-green analysis, but evident of extraction is apparent and the trend is established.

Table 5

LCM Results for Control and Polymer/Cyclohexane Samples

Sample	Cap #	Shots attempted	Shots capture eff.%	Estimated cells captured (visual)	Estimated Non-specific cells (visual)	Extraction eff.% (visual)
Control (no polyslip coating)	1B*	50	100%	70	>150	(not performed)
	2B	50	100%	80	100	
	3B	75	99%	128	>500	
	4B	75	99%	121	>500	
	total	250	99%	399	>1250	
Polyester-Wax in Cyclohexane with glider	1B	75	27%	22	0	100%
	2B	75	13%	10	0	100%
	3B	75	28%	24	0	100%
	4B	75	40%	34	0	100%
	total	300	27%	90	0	100%
Paraffin in Cyclohexane with glider	1B	75	77%	79	52	100%
	2B	75	75%	65	22	100%
	3B	75	88%	84	10	100%
	4B	75	79%	71	31	100%
	total	300	80%	299	115	100%
Polyester-Wax in Cyclohexane with spinner	1B	75	37%	32	45	(not performed)
	2B	75	47%	38	45	
	3B	75	21%	18	39	
	4B	75	16%	12	95	
	total	300	30%	100	224	
Paraffin in Cyclohexane with spinner	1B	75	71%	69	30	(not performed)
	2B	75	87%	75	100	
	3B	75	85%	80	100	
	4B	75	88%	84	100	
	total	300	83%	308	330	

* B = caps manufactured with automated method

** These caps were not extracted due to low shot capt. eff.% and/or high non-specific cells

Table 6

LCM Results for Polymer/Xylene Samples

Sample	Cap #	Shots attempted	Shots capture eff.%	Estimated cells captured (visual)	Estimated Non-specific cells (visual)	Extraction eff.% (visual)
Polyester-Wax in Xylene with glider	1B*	75	43%	39	0	100%
	2B	75	71%	57	30	100%
	3B	75	56%	51	15	100%
	4B	75	47%	49	20	100%
	total	300	54%	196	65	100%
Paraffin in Xylene with glider	1B	75	19%	15	100	(not performed)
	2B	75	0%	0	4	
	3B	75	19%	19	30	
	4B	75	0%	0	0	
	total	300	9%	34	134	
Polyester-Wax in Xylene with spinner	1B	75	40%	35	70	100%
	2B	75	72%	58	25	100%
	3B	75	80%	73	2	100%
	4B	75	81%	67	40	100%
	total	300	68%	233	137	100%
Paraffin in Xylene with spinner	1B	75	59%	51	300	(not performed)
	2B	75	52%	55	200	
	3B	75	63%	58	500	
	4B	75	47%	40	200	
	total	300	55%	204	1200	

* B = caps manufactured with automated method

*** These caps were not extracted due to low shot capt. eff.% and/or high non-specific cells

Non Specific Barrier Film

Another embodiment of the invention will now be described. The purpose of this invention is to prevent contaminating material from migrating from the surface of the tissue slide to the Capsure™ device EVA film.

This embodiment includes a thin layer of transfer adhesive (substance) constructed on a release liner. The transfer adhesive can be extruded or solvent coated on to a liner which has its surface adherence controlled to effect a 'release'. The thickness of the substance can be controlled by line speed on a web, the temperature of a chill roll and the screw speed of an extruder, if an extrusion process used. If a knife-over coater process is used, then coat weight and percentage solids loading concentration in the solvent, in conjunction with line speed and coat roll depth can be utilized to keep the thickness of the substance within specification.

The substance which is to form the barrier layer should be chosen to be a compatible polymer, or polymer blend, to mix with the hot melt adhesive (transfer film) used in LCM. An example of this is ELVAX™ 200W, which is an ethyl vinyl acetate (EVA). Other choices would be blends of EVAs, and / or alloys of EVA and LDPE or wax. The melt index and melt temperature would be chosen to interact in a complimentary way with the LCM process.

Referring to FIGS. 10A-10C, a film 100 is made sandwiched between two layers 110, 120 of release liner (e.g., Polyester). In use, one of the layers (e.g., 120) is peeled back and discarded and the upper layer 110 with the film 100 (aka substance, barrier, transfer adhesive) is layered on top of a tissue section 130 located on a microscope slide 140. Gentle pressure and / or heat is then applied to preferentially adhere the film 100 to the tissue 130 and glass 140. The assembly is allowed to cool and the upper liner 110 is then peeled back and discarded. The tissue sample 130 on the slide has been effectively covered with a transfer adhesive barrier layer and is now ready for LCM. In this way, the tissue 130 has been protected from touching the surface of the LCM transfer device (transfer film) by the presence of the barrier film. The invention reduces non-specific transfer. Hence, no contamination from non-

specifically bound tissue is possible. The LCM process is unaffected by the barrier because it miscibly interacts with the transfer film. The barrier goes with the acquired portion of the tissue and the Capsure™ device after the micro-dissection has been performed.

The context of the invention includes laser capture microdissection. The context of the invention also includes handling, purification and/or analysis of biological material obtained via laser capture microdissection.

The invention can also be included in a kit. The kit can include some, or all, of the components that compose the invention. More specifically, the kit can include the ingredients that compose the composition, a container to hold the composition and/or ingredients, and other components of the invention such as dye(s) and/or applicator(s). The kit can also contain instructions for practicing the invention and apparatus for carrying out the invention. Unless otherwise specified, the components (and apparatus and/or instructions) of the kit can be the same as those used in the invention.

The below-referenced U.S. Patent and U.S. Patent Applications disclose embodiments that were satisfactory for the purposes for which they were intended. The entire contents of U.S. Pat. No. 5,985,085 are hereby expressly incorporated by reference into the present application as if fully set forth herein. The entire contents of U.S. Ser. Nos. 08/800,882; 09/018,452; 09/121,691; 09/121,635; 09/058,711; 09/121,677; 09/208,604; 09/538,862; 09/344,612; 08/984,979; and 09/357,423 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

The term approximately, as used herein, is defined as at least close to a given value (e.g., preferably within 10% of, more preferably within 1% of, and most preferably within 0.1% of). The term substantially, as used herein, is defined as at least approaching a given state (e.g., preferably within 10% of, more preferably within 1% of, and most preferably within 0.1% of). The term coupled, as used herein, is defined as connected, although not necessarily directly, and not necessarily mechanically.

While not being limited to any particular performance indicator, preferred embodiments of the invention can be identified one at a time by testing for improved visualization. The test for the presence of improved visualization can be carried out without undue experimentation by the use of a simple and conventional optical microscope experiment. The apparent contrast of detail within the samples can be characterized, both with and without dyes. Another way to seek preferred embodiments one at a time is to test for reduced non-specific pick-up. The test for reduced non-specific pick-up can be carried out without undue experimentation by the use of a post LCM inspection of the size and shape of the portion of the sample that remains adhered to the LCM transfer film. The amount of sample still adhered to the LCM transfer film beyond the spot diameter can be measured (with optional variation of power density), with special attention being given to asymmetric non-specific transfers as undesirable. Another way to seek preferred embodiments one at a time is to test for reduced sample degradation (with optional variation of storage times before LCM). The test for reduced sample degradation can be carried out without undue experimentation by testing the accuracy of diagnostic assays on LCM acquired portions of samples that are known to be positive.

All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the barrierized samples described herein can be physically separate modules, it will be manifest that the barrierized samples may be integrated into

additional apparatus with which they are associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements.

The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A method of processing a biological sample for laser capture microdissection, comprising:
 - providing said biological sample; and
 - applying a substance to said biological sample so as to provide a barrier between said biological sample and a surrounding environment.
2. The method of claim 1, wherein said substance is provided as a liquid and applying said substance includes coating said liquid on said biological sample.
3. The method of claim 2, wherein coating includes at least one technique selected from the group consisting of spraying, dipping and dripping.
4. The method of claim 2, wherein coating includes at least one technique selected from the group consisting of rolling and spin coating.
5. The method of claim 1, wherein said substance is provided as a film and applying said substance includes laminating said film to said biological sample.
6. The method of claim 5, wherein laminating includes melting
7. The method of claim 6, wherein melting includes melting with a heat block.
8. The method of claim 6, wherein melting includes melting with an infrared sweep.

9. The method of claim 1, wherein providing said biological sample includes mounting said biological sample on a glass slide.
10. The method of claim 1, wherein said substance includes a polymer.
11. The method of claim 10, wherein said polymer includes ethyl vinyl acetate.
12. The method of claim 10, wherein said polymer includes polyester.
13. The method of claim 1, wherein said substance includes paraffin.
14. The method of claim 1, wherein said substance includes a solvent.
15. The method of claim 14, wherein said solvent includes xylene.
16. The method of claim 14, wherein said solvent includes cyclohexane.
17. The method of claim 1, wherein said substance includes a dye.
18. The method of claim 1, further comprising laser capture microdissecting said biological sample.
19. A kit to provide a barrier layer on a biological sample that is to undergo laser capture microdissection, comprising:
 - a solvent;
 - and a solute to be dissolved in said solvent.
20. The kit of claim 19, further comprising a container that holds said solvent.

21. The kit of claim 19, further comprising instructions describing preparation of biological samples for laser capture microdissection.
22. An article of manufacture, comprising:
a biological sample that is to undergo laser capture microdissection; and
a barrier coupled to at least a portion of said biological sample.
23. The article of manufacture according to claim 22, wherein said barrier includes a polymer.
24. The article of manufacture according to claim 22, wherein said polymer includes ethyl vinyl acetate.
25. The article of manufacture according to claim 22, wherein said polymer includes polyester.
26. The article of manufacture according to claim 22, wherein said barrier includes paraffin.
27. The article of manufacture according to claim 22, wherein said barrier includes a dye.
28. An article of manufacture, comprising:
a portion of a biological sample that has undergone laser capture microdissection; and
a barrier coupled to said portion of said biological sample.
29. The article of manufacture according to claim 28, further comprising a laser capture microdissection film coupled to said barrier.

30. The article of manufacture according to claim 29, further comprising a laser capture microdissection film carrier coupled to said laser capture microdissection film.
31. The article of manufacture according to claim 28, wherein said barrier includes a polymer.
32. The article of manufacture according to claim 28, wherein said polymer includes ethyl vinyl acetate.
33. The article of manufacture according to claim 28, wherein said polymer includes polyester.
34. The article of manufacture according to claim 28, wherein said barrier includes paraffin.
35. The article of manufacture according to claim 28, wherein said barrier includes a dye.
36. A composition to process a biological sample for laser capture microdissection, comprising:
a solvent; and
solute in said solvent, said solute capable of forming a barrier on said biological sample.
37. The composition of claim 36, wherein said solute includes a polymer.
38. The composition of claim 37, wherein said polymer includes at least one member selected from the group consisting of ethyl vinyl acetate and polyester.

39. The composition of claim 36, adapted for doctor blading, wherein said solute includes 2.5% by composition weight paraffin and said solvent includes cyclohexane.
40. The composition of claim 36, wherein said solute includes paraffin.
41. The composition of claim 36, wherein said solvent includes xylene.
42. The composition of claim 36, adapted for spin coating, wherein said solute includes 5.0% by composition weight paraffin and said solvent includes cyclohexane.
43. The composition of claim 1, further comprising a dye.
44. A method, comprising:
applying a substance to at least a portion of a biological sample that is to undergo laser capture microdissection.
45. The method of claim 44, further comprising:
contacting at least a portion of said substance with a laser capture microdissection transfer film; and
separating a fraction of said sample from a remainder of said sample.
46. The method of claim 45, wherein applying includes coating.
47. The method of claim 44, wherein said substance is applied to at least said portion of said sample as a liquid layer and then cured.
48. The method of claim 47, wherein applying includes laminating.

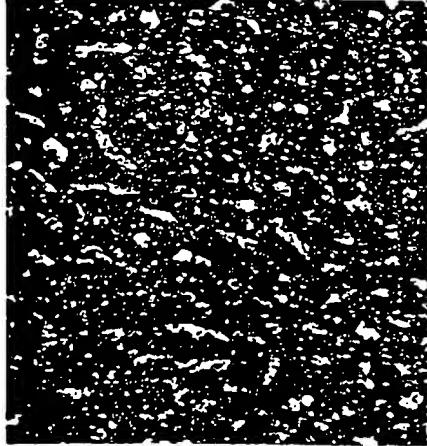
49. The method of claim 48, wherein said substance is applied to at least said portion of said sample as a solid layer having a thickness which is not substantially altered during the step of applying.
50. An apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising:
a container adapted to provide a fluid source of said substance, said container including an orifice that defines a principal plane that is substantially parallel to a primary direction of movement that is to be taken by said biological sample while said substance is being applied.
51. The apparatus of claim 50, further comprising a receptacle coupled to said orifice, said receptacle recirculating excess amounts of said substance to said container.
52. An apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising:
a release layer; and
a solid layer of said substance coupled to said release layer.
53. The apparatus of claim 52, further comprising another release layer coupled to said solid layer.
54. An apparatus to apply a substance to at least a portion of a biological sample that is to undergo laser capture microdissection, comprising:
a blade including an indexing surface and a surface to define a gap that defines a principal plane that is held at an acute angle with respect to a perpendicular to a primary direction of movement that is to be taken by said biological sample while said substance is being applied.

55. The apparatus of claim 54, further comprising a container adapted to provide a fluid source of said substance, said container coupled to said blade.

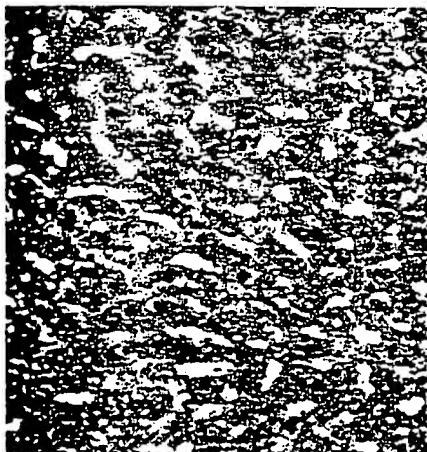
ABSTRACT OF THE DISCLOSURE

Systems and methods are described for barriers on laser capture microdissection samples. A method of processing a biological sample for laser capture microdissection, includes: providing the biological sample; and applying a substance to the biological sample so as to provide a barrier between the biological sample and a surrounding environment. The systems and methods provide advantages because non-specific pick-up is reduced, visualization is improved, sample degradation is reduced, and contamination is reduced.

F16 1A
prior art



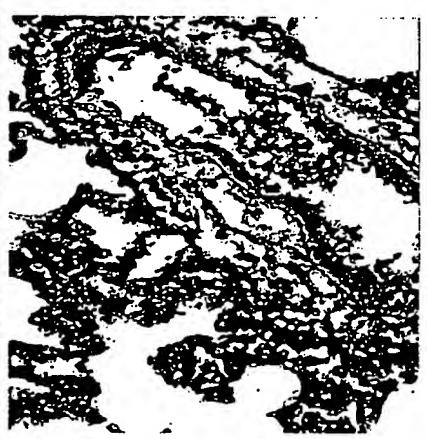
F16 2A
prior art



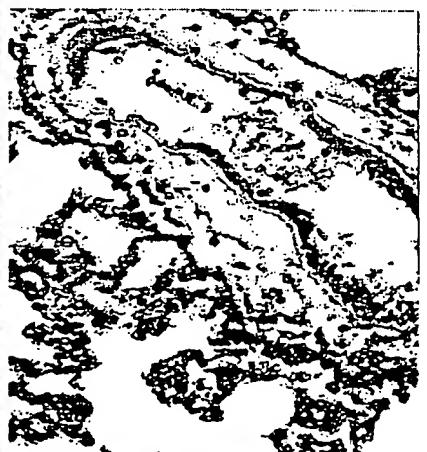
F16 3A



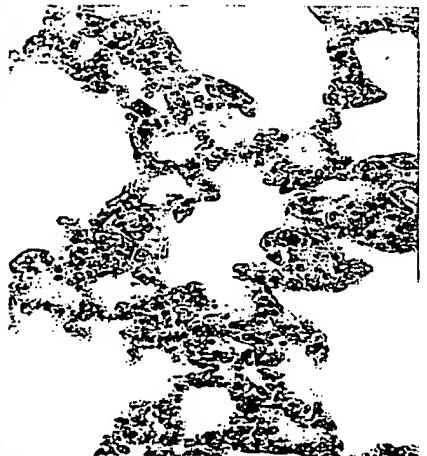
F16 1B
prior art



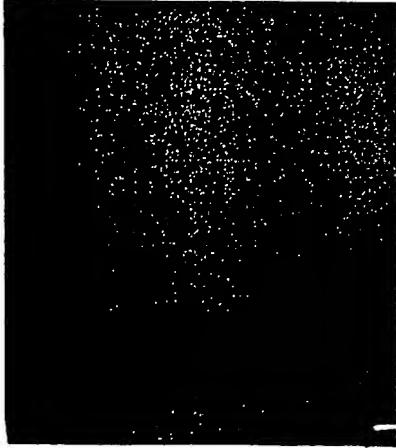
F16 2B
prior art



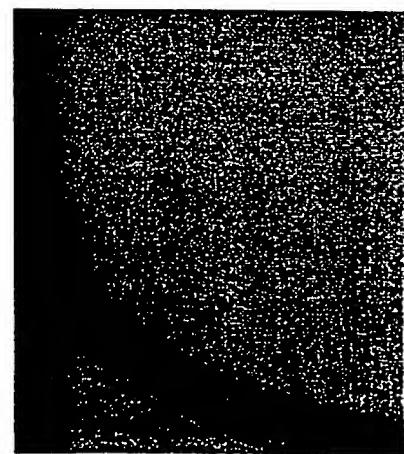
F16 3B



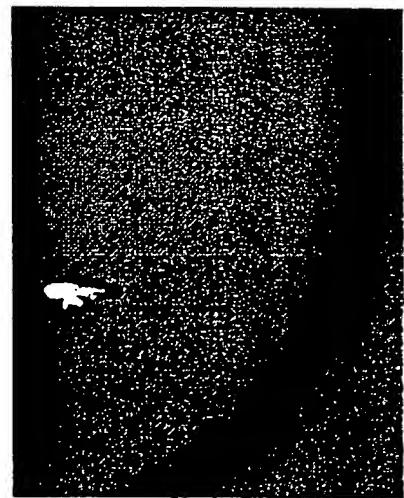
F16 4A
prior art



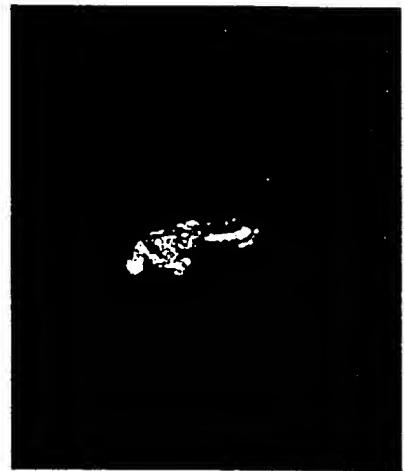
F16 4B
prior art



F16 5A



F16 5B



F16 5C

F16 6A
prior art



F16 6B
prior art



F16 6C
prior art



F16 7A



F16 7B



F16 7C

FIG. 8.

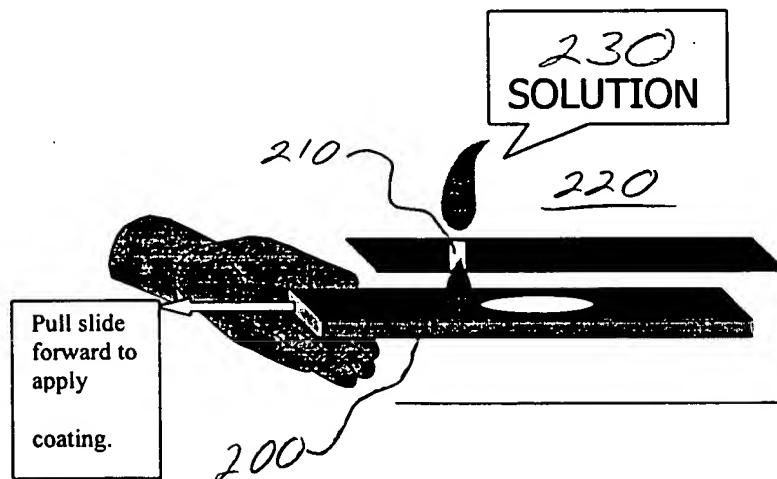
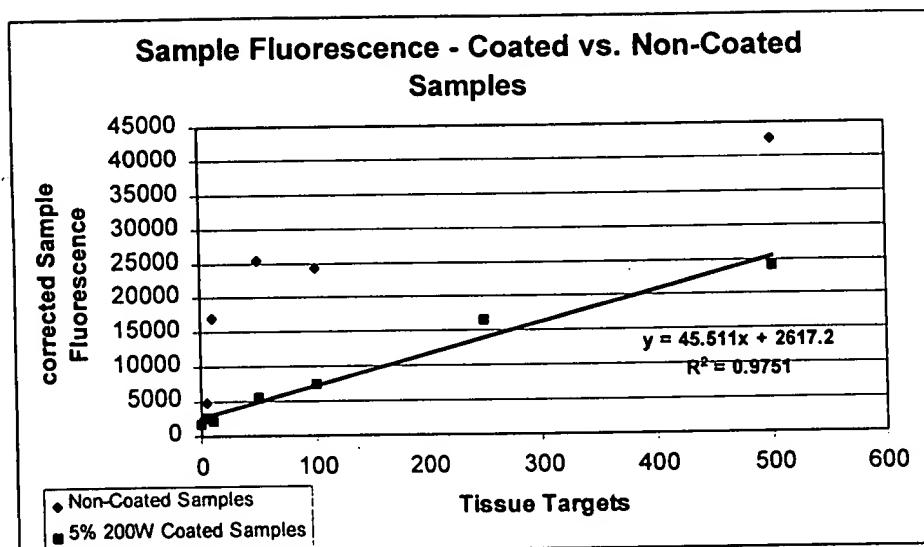


FIG. 9

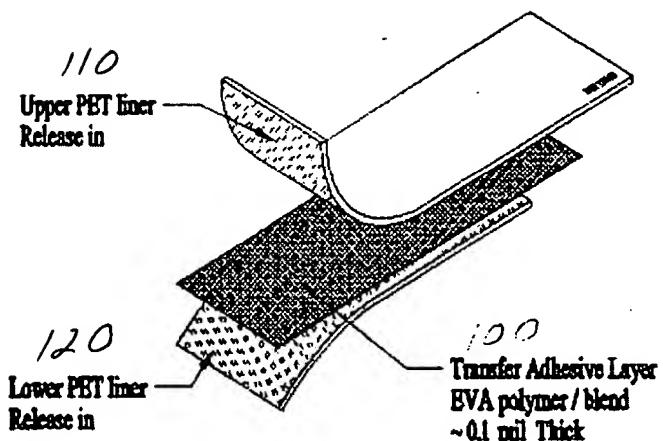


FIG 10A

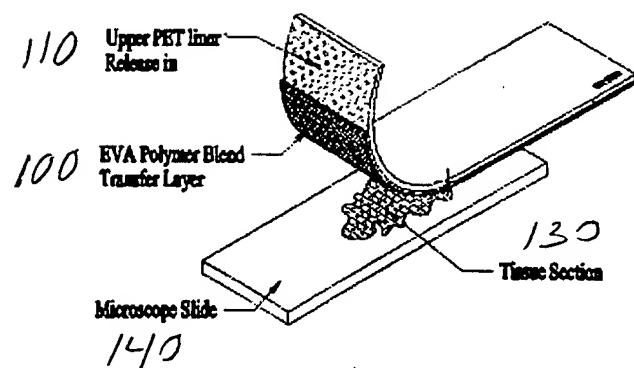


FIG 10B

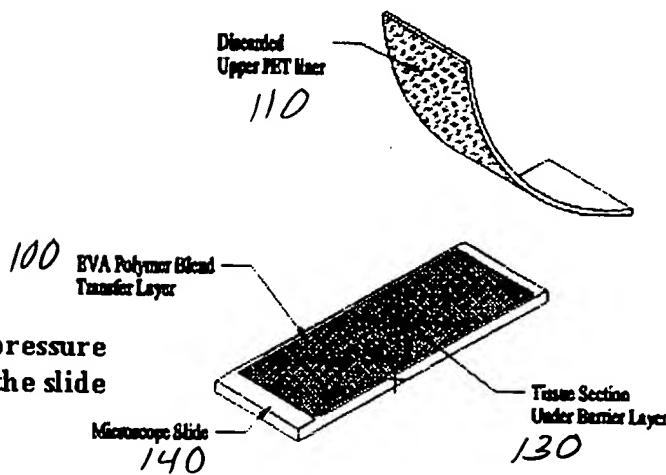


FIG 10C

FIG 11A

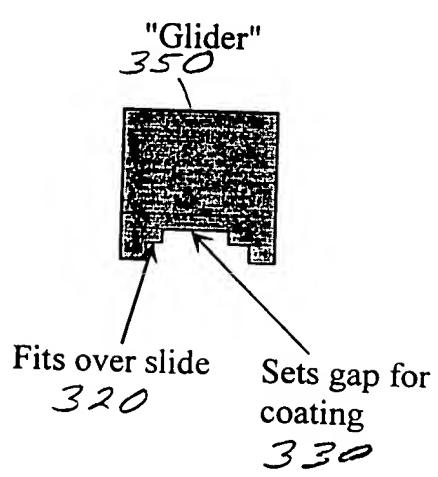
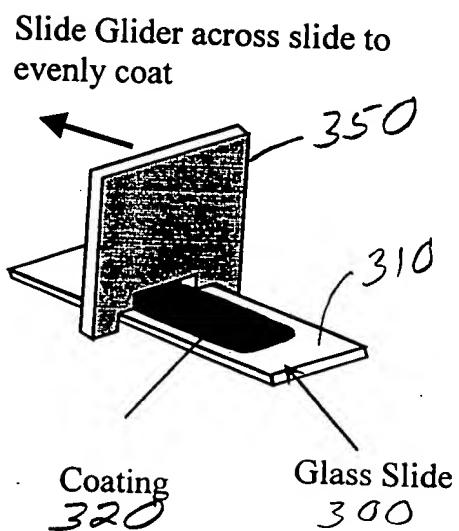


FIG 11B



09/617,742

UNITED STATES PATENT APPLICATION
ENTITLED

**LASER CAPTURE MICRODISSECTION
OPTICAL SYSTEM**

Inventors: Thomas M. Baer, Mark A. Enright,
David F. Head, and Christopher E. Todd

Citizenship: All of the United States

PATENT
Attorney Docket No. 17726-705

**LASER CAPTURE MICRODISSECTION
OPTICAL SYSTEM**

Inventors: Thomas M. Baer; Mark A. Enright
David F. Head; and Christopher E. Todd

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is, under 35 U.S.C. § 120, a continuation of U.S. Ser. No. 09/018,452, filed February 4, 1998, now pending, which is in-turn 5 a continuation-in-part of both U.S. Ser. No. 60/060,731, filed October 1, 1997, now pending, and U.S. Ser. No. 60/037,864, filed February 7, 1997, now abandoned, the entire contents of all which are hereby incorporated herein by reference as if fully set forth herein.

10

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to the field of laser capture 15 microdissection (LCM). More particularly, the invention relates to inverted microscopes that include specialized apparatus for performing LCM. Specifically, a preferred implementation of the invention relates to an inverted microscope that includes a cap handling subsystem, an illumination/laser optical subsystem, a vacuum chuck subsystem, and a

manual joystick subsystem. The invention thus relates to inverted microscopes of the type that can be termed laser capture microdissection inverted microscopes.

5

Discussion of the Related Art

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from 10 the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

15

A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene 20 vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

25

In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the

tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes
5 localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue
10 section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the
15 tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ
20 breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

25 Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research.

For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples
5 from tissue samples.

The LCM technique is generally described in the recently published article: *Laser Capture Microdissection*, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM
10 technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.
15

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed.
20 The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.
25

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

5 The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is
10 mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

15 While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

20 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for laser capture microdissection. There is also a particular need for an improved method of laser capture microdissection.

A first aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection method, comprising: providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument; providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface; placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and then transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.

A second aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection instrument, comprising: an inverted microscope including: an illumination/laser optical subsystem; a translation stage coupled to said illuminator/laser optical subsystem; a cap handling subsystem coupled to said translation stage; a vacuum chuck subsystem coupled to said translation stage; and a manual joystick subsystem coupled to said translation stage.

5

These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

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BRIEF DESCRIPTION OF THE DRAWINGS

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A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention;

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1;

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

5 FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention;

10 FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention;

FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6;

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention;

15 FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8;

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention;

20 FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10;

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention;

25 FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention;

FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention;

5 FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention;

10 FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention;

15 FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention; and

20 FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

15

DESCRIPTION OF PREFERRED EMBODIMENTS

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The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

25

The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997 entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed

October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as

polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is 5 picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be 10 manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement. Further, this simultaneous movement can be effected with in a single 15 handed manner. The acquisition of samples is thus made quicker.

Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication 20 of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a function of operator hand movement.

In addition, the joystick provides tactile feedback not available with 25 electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X

and Y) as a function of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

5 Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185.
10 After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.
15

20 Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be
25 appreciated from FIG. 2B that the illumination beam path includes delivery

of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which can include a camera. In idle mode, the laser beam path 310 provides a visible low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second, preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength

of the laser is 810 nannometers. In a preferred embodiment the spot size of
the laser at the EVA material located on microcentrifuge tube cap 120 is
variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more
preferably from 5 to 30 microns. These ranges are relatively preferred when
5 designing the optical subsystem. From the standpoint of the clinical
operator, the widest spot size range is the most versatile. A lower end point
in the spot size range on the order of 5 microns is useful for transferring
single cells.

10 Suitable lasers can be selected from a wide power range. For
example, a 100 watt laser can be used. On the other hand, a 50 mW laser
can be used. The laser can be connected to the rest of the optical subsystem
with a fiber optical coupling. Smaller spot sizes are obtainable using
diffraction limited laser diodes and/or single mode fiber optics. Single
mode fiber allows a diffraction limited beam.

15 While the laser diode can be run in a standard mode such as TEM_{00} ,
other intensity profiles can be used for different types of applications.
Further, the beam diameter could be changed with a stepped lens instead of
lens 350.

20 Changing the beam diameter permits the size of the portion of the
sample that is acquired to be adjusted. Given a tightly focused initial
condition, the beam size can be increased by defocusing. Given a defocused
initial condition, the beam size can be decreased by focusing. The change in
focus can be in fixed amounts. The change in focus can be obtained by
means of indents on a movable lens mounting and/or by means of optical
25 glass steps. In any event, increasing/decreasing the optical path length is the
effect that is needed to alter the focus of the beam, thereby altering the spot

size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic), and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial. Fluorescence emitted by the sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for damping vertical motion of the cap handling mechanic subassembly 110.

5 The dampener 510 is adapted to lower the microcentrifuge tube cap 120 down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable of retarding the vertical motion of the subassembly 110 so as not to generate an impulse. As

10 the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before

15 the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to

20 microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube

25 cap 120 and then the microcentrifuge tube cap 120 is picked up off of the

slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is

5

lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 off the slide (not shown).

10

The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture microdissection transfer film is approximately 0.26 square inches.

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

15

Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to the position depicted in FIGS. 6-7).

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Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

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Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

25

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and

the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This
5 translation capability is present when the vacuum is off and when the vacuum is on.

There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place.

Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the
10 glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is
15 connected to the manifold holes 1020-1030. The manifold holes 1020-1030 are coupled with the manifold holes 1020-1030.

It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top
20 surface without constraint.

Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430
25 collimates the light from the fiber optic 1410. The collimator lens 1430 can

be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens). A collimated beam 1440 from the collimator lens 1430 then passes through a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460 is coaxial with the white light illumination. Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG 010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470 can have a numerical aperture of approximately 0.8. This can be characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that contains the cells to be sampled.

More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type eliminates the need for refractive index matching of the sample. Such a scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk or opal glass which is

a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example, frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser.

5 In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light

10 sufficiently.

Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed

15 into the microcentrifuge tube cap 120 so as to acquire the desired sample.

The purpose of the illuminator design is to provide a very high numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization,

20 this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important

25 because the sample is adjacent the bottom of the sample carrier and the

objective cannot move closer to the sample than the top of the sample carrier.

5 The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in visualization. This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

10 The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

15 In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

20 Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

25 The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a

speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is

pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the velocity should be such that the sample is thixotropically sheared.

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Practical Applications of the Invention

A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases.
10 This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient
15 samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually
20 innumerable uses for the invention, all of which need not be detailed here.

Advantages of the Invention

A laser capture microdissection instrument and/or method
25 representing an embodiment of the invention can be cost effective and advantageous for at least the following reasons. The invention will replace

current methods with better technology that allows for more accurate and reproducible results. The invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

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All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent was specifically and individually indicated to be incorporated in its entirety

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by reference.

All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

15

For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the

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LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

10

It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
 - providing a sample that is to undergo laser capture microdissection;
 - positioning said sample within an optical axis of a laser capture microdissection instrument, said laser capture microdissection instrument including an illumination/laser beam delivery system;
 - providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface;
 - placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; then
 - illuminating said sample with said illumination/laser beam delivery system; and then
 - transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film, with said illumination/laser beam delivery system.
2. The method of claim 1, wherein said illumination/laser beam delivery system includes a white light illuminator and the step of illuminating said sample includes illuminating said sample with said white light illuminator.

3. The method of claim 2, wherein illuminating said sample with said white light illuminator includes passing white light toward said transfer film carrier through both a dichroic mirror and a focusing lens.
4. The method of claim 3, further comprising superimposing a beam from a laser with white light illumination from said white light illuminator.
5. The method of claim 1, wherein said illumination/laser beam delivery system includes a laser capture microdissection optical train and the step of transferring a portion of said sample includes transferring a portion of said sample with said laser capture microdissection optical train.
6. The method of claim 5, wherein transferring a portion of said sample with said laser capture microdissection optical train includes reflecting a collimated beam with a beam steering mirror and then reflecting said collimated beam with a dichroic mirror through a focusing lens toward said transfer film carrier.
7. The method of claim 6, further comprising adjusting a beam spot size with said focusing lens, said beam spot size being defined by said collimated beam.
8. The method of claim 6, further comprising changing a beam diameter with a variable aperture, said beam diameter being defined by said collimated beam.

9. The method of claim 6, further comprising passing said collimated beam through an objective and then reflecting said collimated beam to a cut-off filter.
10. The method of claim 5, further comprising superimposing a beam from said laser capture microdissection optical train with white light illumination from a white light illuminator.
11. The method of claim 1, further comprising delivering optical information to an image acquisition system with said illumination/laser beam delivery system.
12. The method of claim 1, further comprising delivering optical information to an eyepiece assembly with said illumination/laser beam delivery system.
13. The method of claim 1, wherein said sample includes a fluorescent system, and, further comprising exciting said fluorescent system.
14. The method of claim 13, further comprising identifying at least a portion of said sample with light that excites said fluorescent system, before the step of transferring.
15. The method of claim 1, wherein illuminating said sample includes condensing a collimated beam of illumination light.

16. The method of claim 15, wherein illuminating said sample includes passing said collimated beam of illumination light through a beam splitter.
17. The method of claim 16, wherein transferring said portion of said sample includes injecting a laser beam by reflecting said laser beam with said beam splitter.
18. The method of claim 15, wherein said collimated beam of illumination light is obtained by collimating a diverging beam of illumination light with an aspheric lens.
19. The method of claim 18, wherein said diverging beam of illumination light is obtained from a fiber optic.
20. The method of claim 1, wherein the step of illuminating said sample includes scattering illumination light with a scattering media.
21. The method of claim 20, wherein scattering illumination light with said scattering media includes scattering illumination light with said transfer film carrier.
22. A laser capture microdissection instrument, comprising:
an illumination/laser beam delivery system.

23. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a white light illuminator.
24. The laser capture microdissection instrument of claim 23, wherein said illumination/laser beam delivery system includes a dichroic mirror optically coupled to said while light illuminator and a focusing lens optically coupled to said dichroic mirror.
25. The laser capture microdissection instrument of claim 24, wherein said illumination/laser beam delivery system includes a laser diode optically coupled to said focusing lens.
26. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a laser capture microdissection optical train.
27. The laser capture microdissection instrument of claim 26, wherein said laser capture microdissection optical train includes a laser, a beam steering mirror optically coupled to said laser, a dichroic mirror optically coupled to said beam steering mirror, and a focusing lens optically coupled to said dichroic mirror.
28. The laser capture microdissection instrument of claim 27, wherein said laser capture microdissection optical train includes an objective

optically coupled to said focusing lens and a cut-off filter optically coupled to said objective.

29. The laser capture microdissection instrument of claim 27, wherein said laser capture microdissection optical train includes a variable aperture optically coupled to said focusing lens.

30. The laser capture microdissection instrument of claim 27, wherein said laser capture microdissection optical train includes a stepped prism that can be optically coupled to said focusing lens.

31. The laser capture microdissection instrument of claim 27, wherein said illumination/laser beam delivery system includes a white light illuminator optically coupled to said dichroic mirror.

32. The laser capture microdissection instrument of claim 22, further comprising an image acquistion system optically coupled to said illumination/laser beam delivery system.

33. The laser capture microdissection instrument of claim 22, further comprising an eyepiece assembly optically coupled to said illumination/laser beam delivery system.

34. The laser capture microdissection instrument of claim 22, further comprising a fluorescent system optically coupled to said illumination/laser beam delivery system.

35. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a condensing lens.
36. The laser capture microdissection instrument of claim 35, wherein said illumination/laser beam delivery system includes a beam splitter optically coupled to said condensing lens.
37. The laser capture microdissection instrument of claim 36, wherein said illumination/laser beam delivery system includes a laser optically coupled to said beam splitter.
38. The laser capture microdissection instrument of claim 37, wherein said illumination/laser beam delivery system includes a fiber optic optically coupled to said beam splitter.
39. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a scattering media.
40. The laser capture microdissection instrument of claim 39, wherein said scattering media includes a transfer film carrier.
41. The laser capture microdissection instrument of claim 22, further comprising a translation stage coupled to said illumination/laser beam delivery system.

42. The laser capture microdissection instrument of claim 41, further comprising a manual joystick subsystem connected to said translation stage.
43. The laser capture microdissection instrument of claim 41, further comprising a vacuum chuck subsystem connected to said translation stage.
44. The laser capture microdissection instrument of claim 22, further comprising a transfer film carrier handling subsystem coupled to said illumination/laser beam delivery system.
45. An inverted microscope, comprising:
an illumination/laser beam delivery system.
46. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a white light illuminator.
47. The inverted microscope of claim 46, wherein said illumination/laser beam delivery system includes a dichroic mirror optically coupled to said white light illuminator and a focusing lens optically coupled to said dichroic mirror.
48. The inverted microscope of claim 47, wherein said illumination/laser beam delivery system includes a laser diode optically coupled to said focusing lens.

49. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a laser capture microdissection optical train.
50. The inverted microscope of claim 49, wherein said laser capture microdissection optical train includes a laser, a beam steering mirror optically coupled to said laser, a dichroic mirror optically coupled to said beam steering mirror, and a focusing lens optically coupled to said dichroic mirror.
51. The inverted microscope of claim 50, wherein said laser capture microdissection optical train includes an objective optically coupled to said focusing lens and a cut-off filter optically coupled to said objective.
52. The inverted microscope of claim 50, wherein said laser capture microdissection optical train includes a variable aperture optically coupled to said focusing lens.
53. The inverted microscope of claim 50, wherein said laser capture microdissection optical train includes a stepped prism that can be optically coupled to said focusing lens.
54. The inverted microscope of claim 50, wherein said illumination/laser beam delivery system includes a white light illuminator optically coupled to said dichroic mirror.

55. The inverted microscope of claim 45, further comprising an image acquisition system optically coupled to said illumination/laser beam delivery system.
56. The inverted microscope of claim 45, further comprising an eyepiece assembly optically coupled to said illumination/laser beam delivery system.
57. The inverted microscope of claim 45, further comprising a fluorescent system optically coupled to said illumination/laser beam delivery system.
58. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a condensing lens.
59. The inverted microscope of claim 58, wherein said illumination/laser beam delivery system includes a beam splitter optically coupled to said condensing lens.
60. The inverted microscope of claim 59, wherein said illumination/laser beam delivery system includes a laser optically coupled to said beam splitter.
61. The inverted microscope of claim 60, wherein said illumination/laser beam delivery system includes a fiber optic optically coupled to said beam splitter.

62. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a scattering media.
63. The inverted microscope of claim 62, wherein said scattering media includes a transfer film carrier.
64. The inverted microscope of claim 45, further comprising a translation stage coupled to said illumination/laser beam delivery system.
65. The inverted microscope of claim 64, further comprising a manual joystick subsystem connected to said translation stage.
66. The inverted microscope of claim 64, further comprising a vacuum chuck subsystem connected to said translation stage.
67. The inverted microscope of claim 45, further comprising a transfer film carrier handling subsystem coupled to said illumination/laser beam delivery system.

ABSTRACT OF THE DISCLOSURE

Systems and methods for laser capture microdissection are disclosed.

An inverted microscope includes an illumination/laser beam delivery system
that is adapted to both illuminate a sample and provide energy for laser
capture microdissection of the sample. The systems and methods provide
the advantages of increased speed and much lower rates of contamination.
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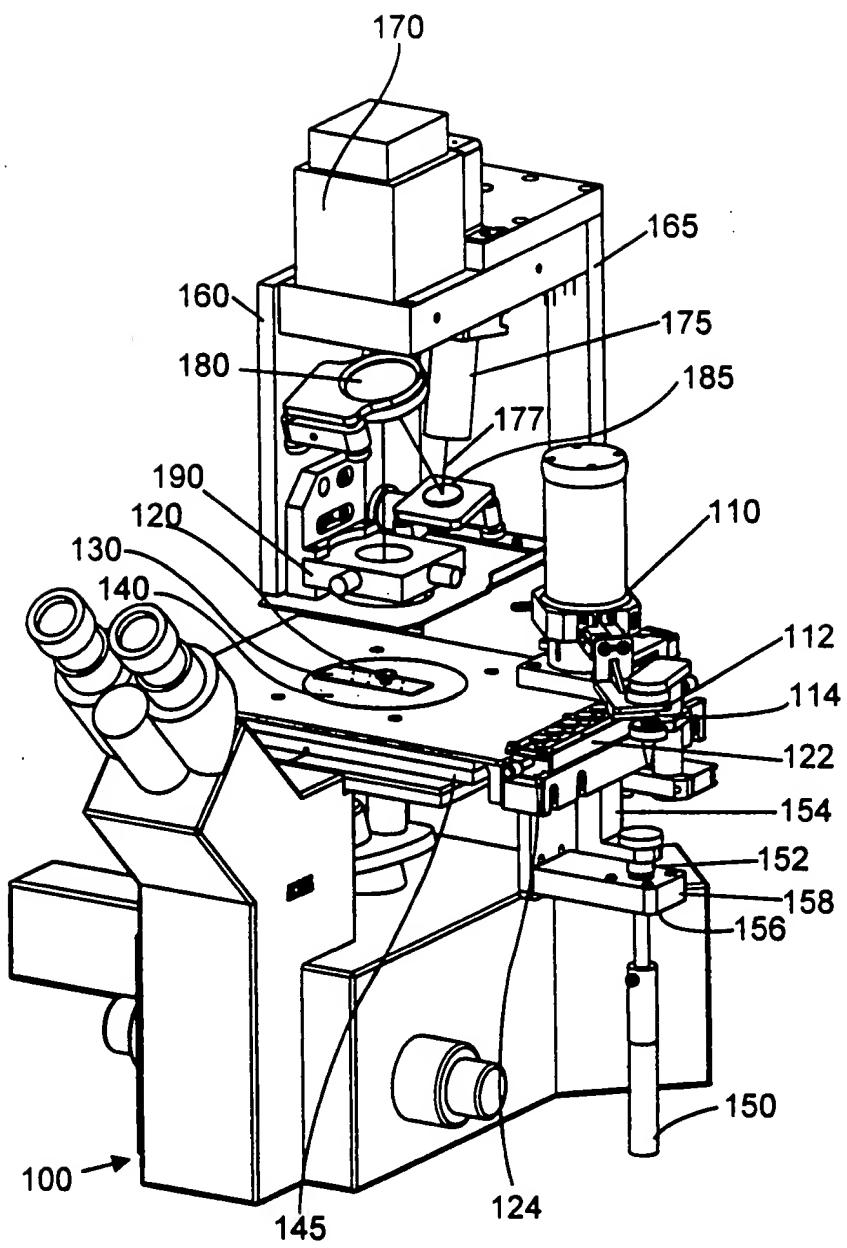
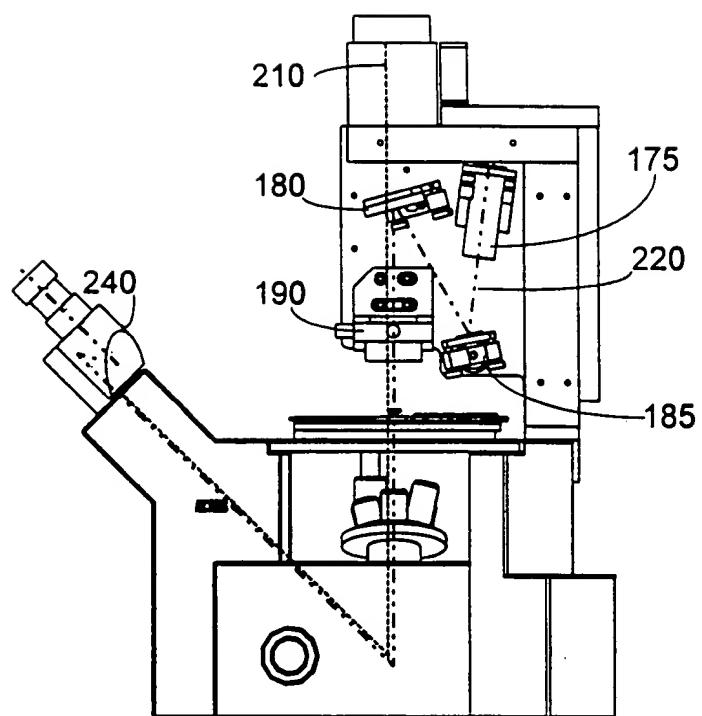
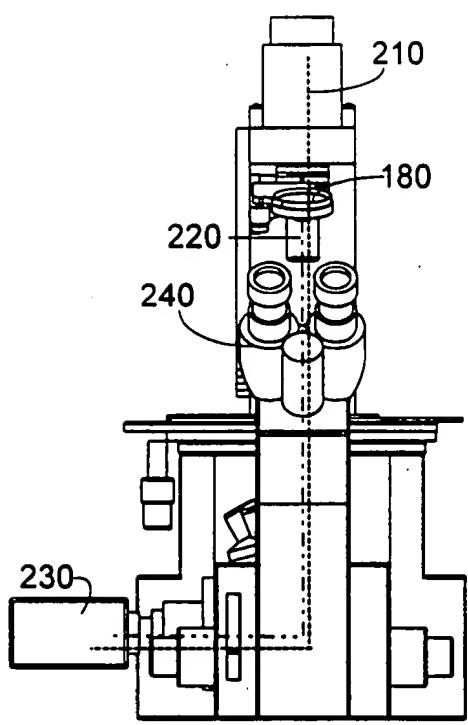


FIG. 1



— WHITE LIGHT ILLUMINATION PATH
— LASER BEAM PATH

FIG. 2A

FIG. 2B

FIG. 3

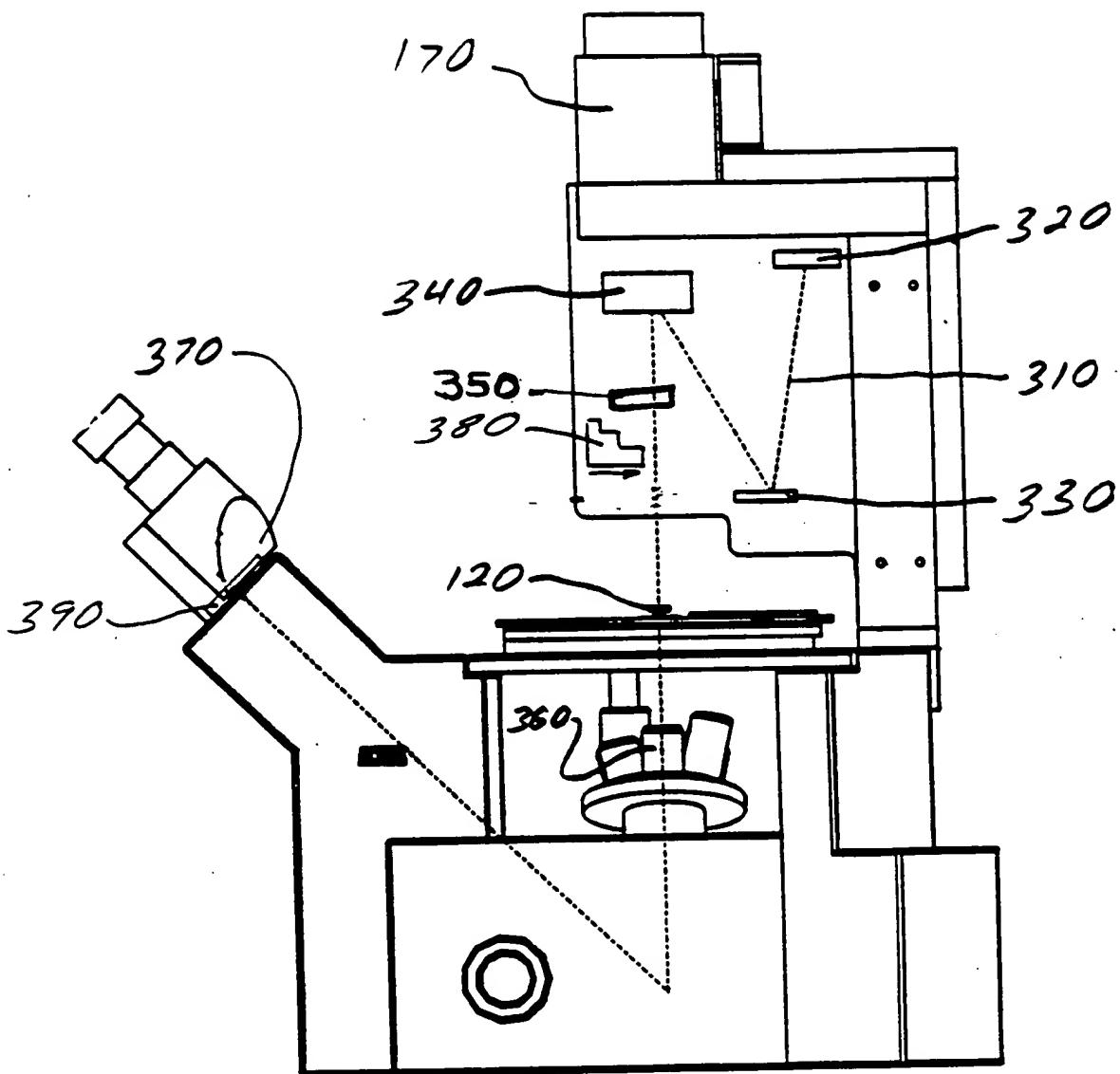
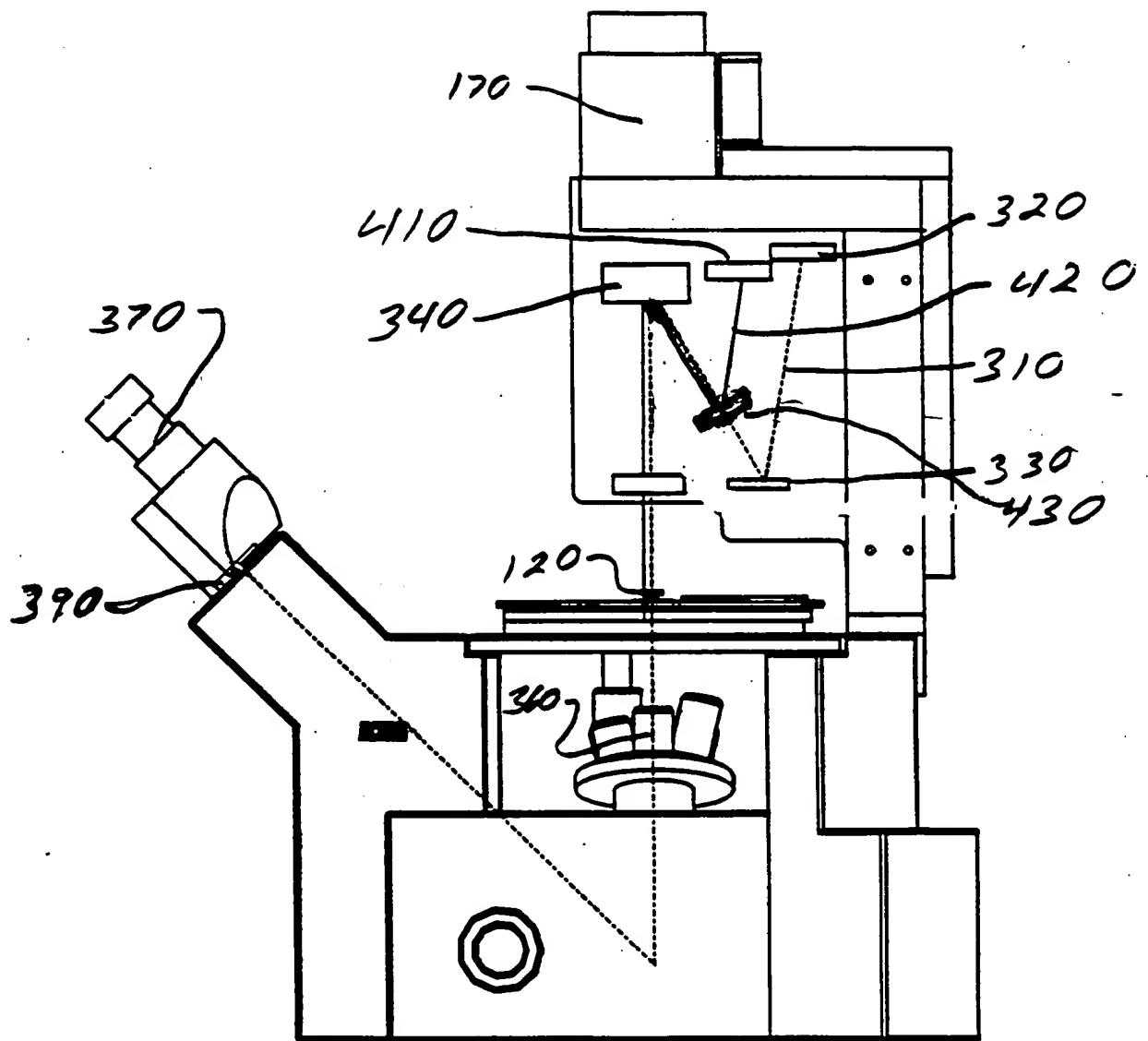


FIG. 4



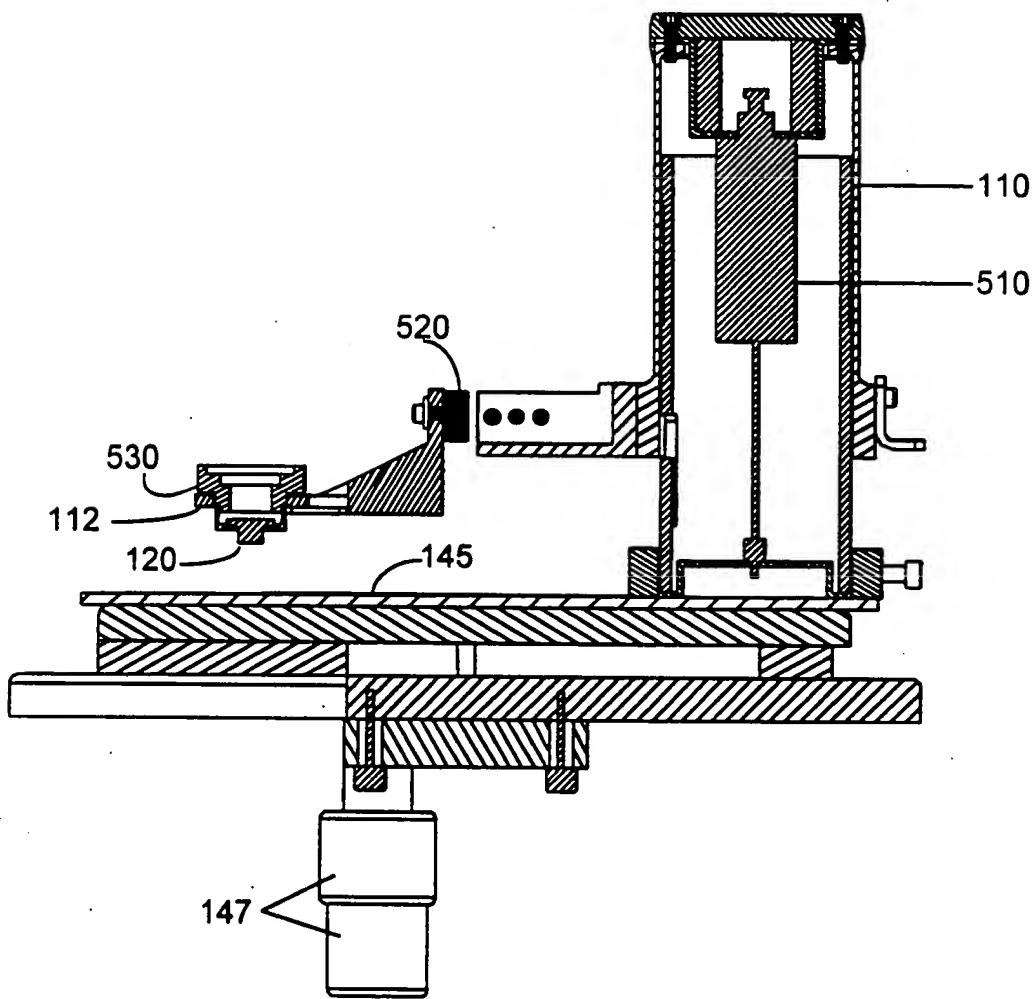


FIG. 5

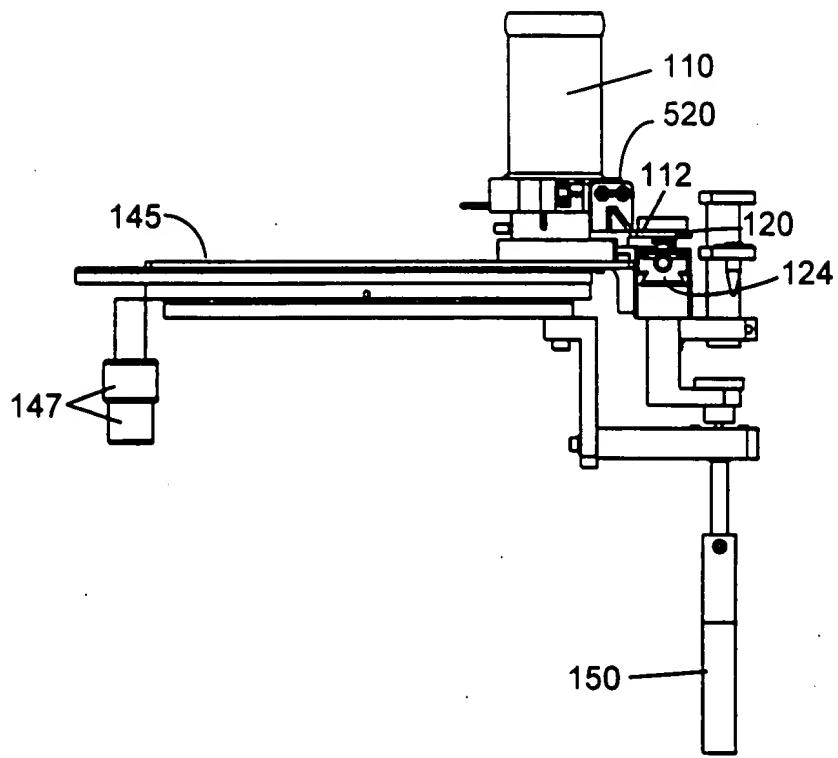


FIG. 6

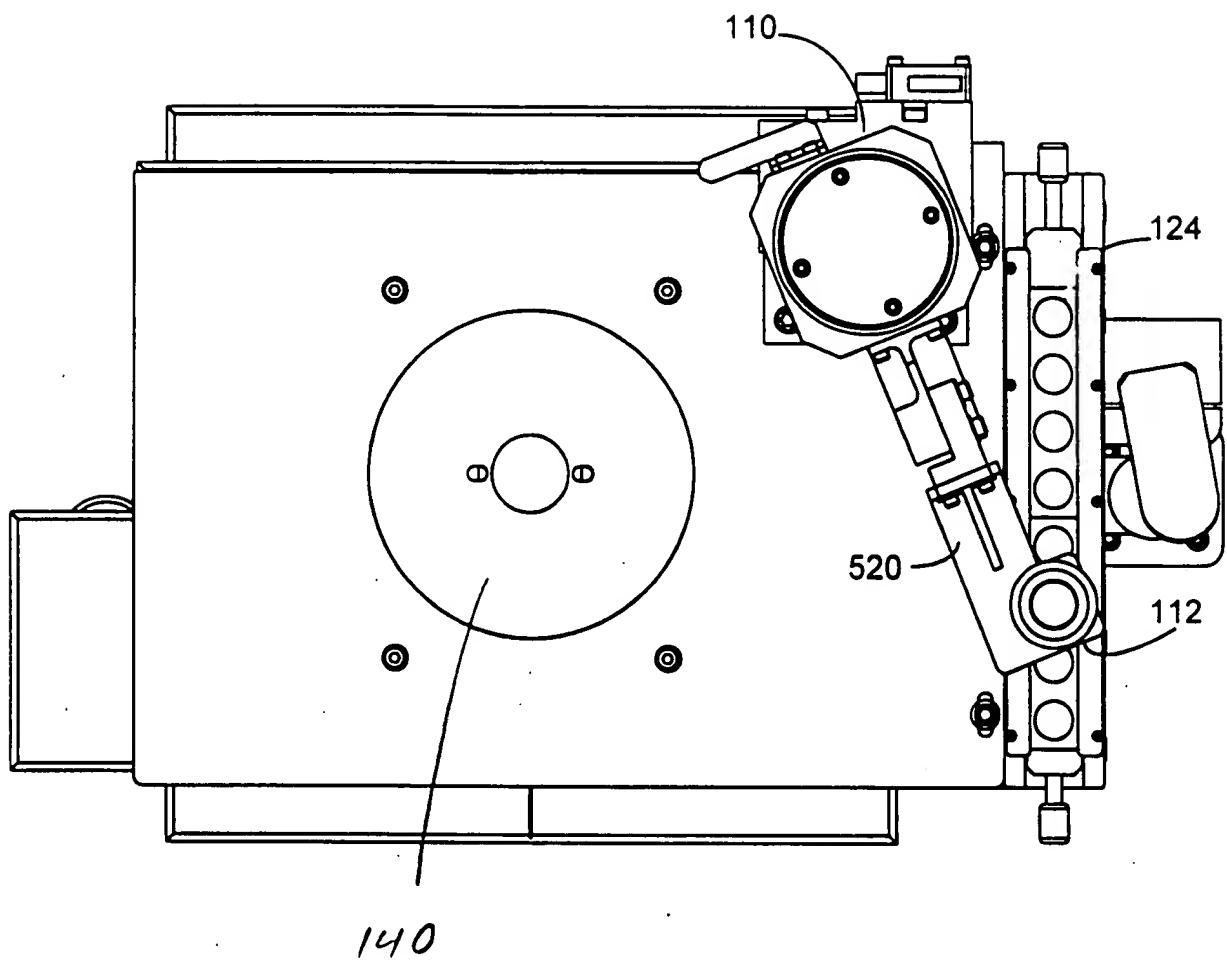


FIG. 7

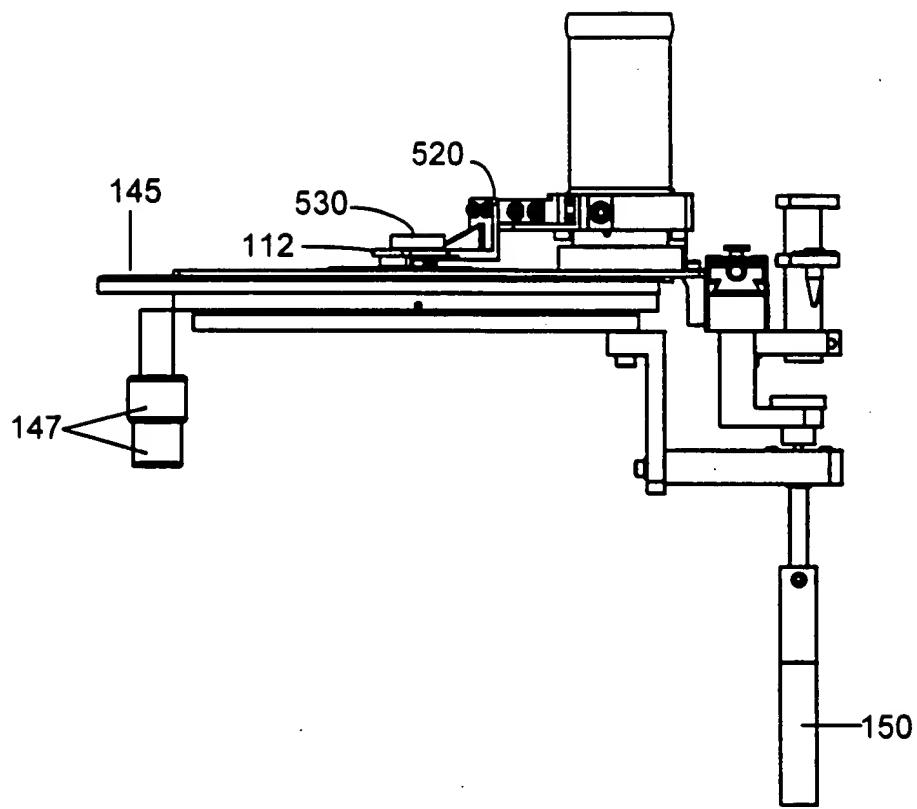


FIG. 8

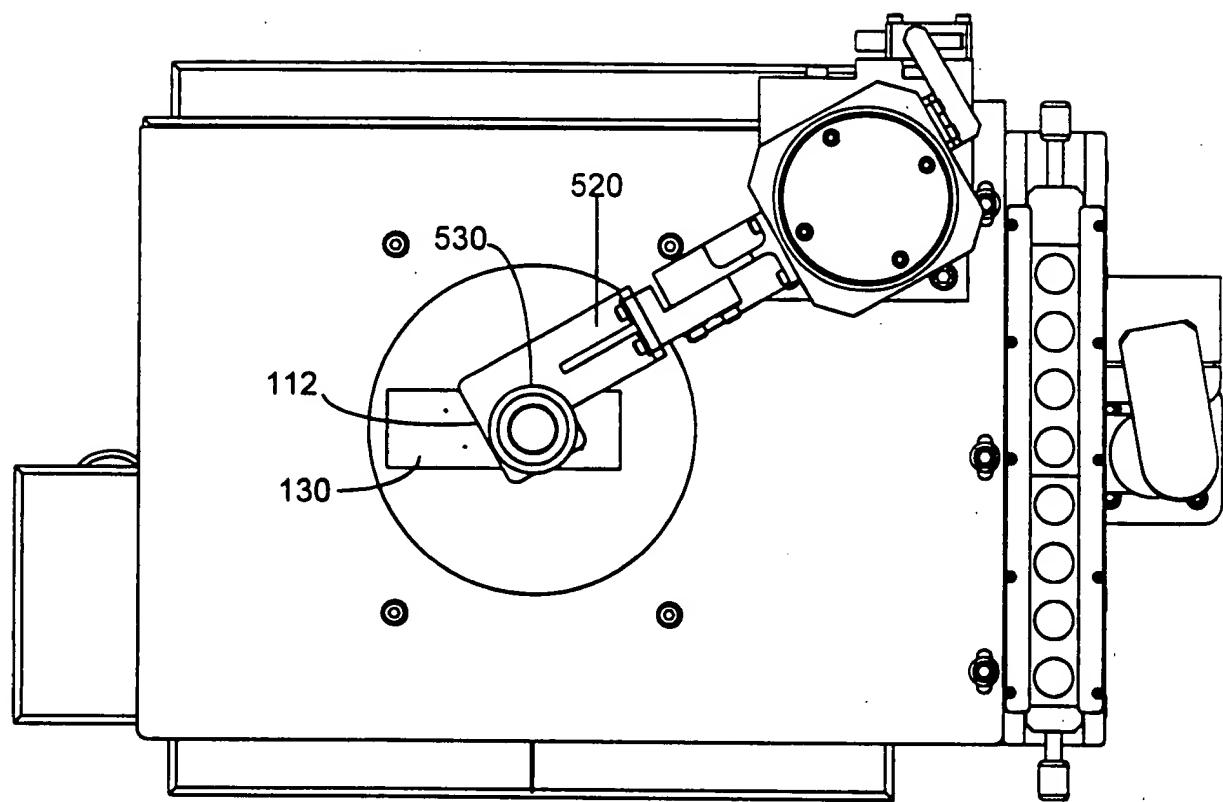


FIG. 9

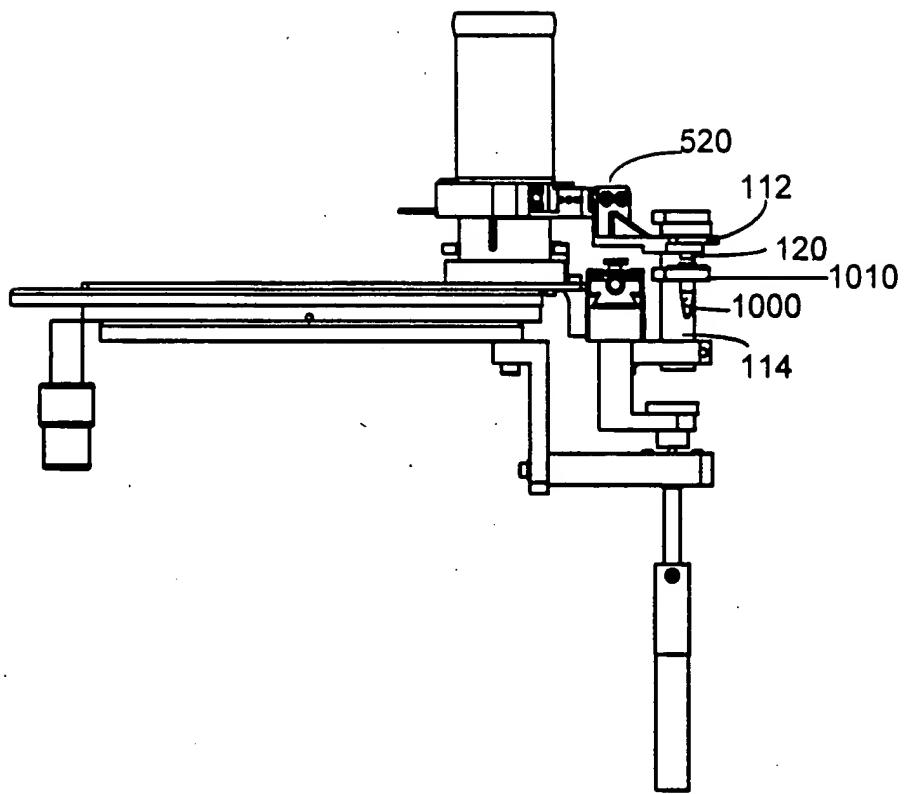


FIG. 10

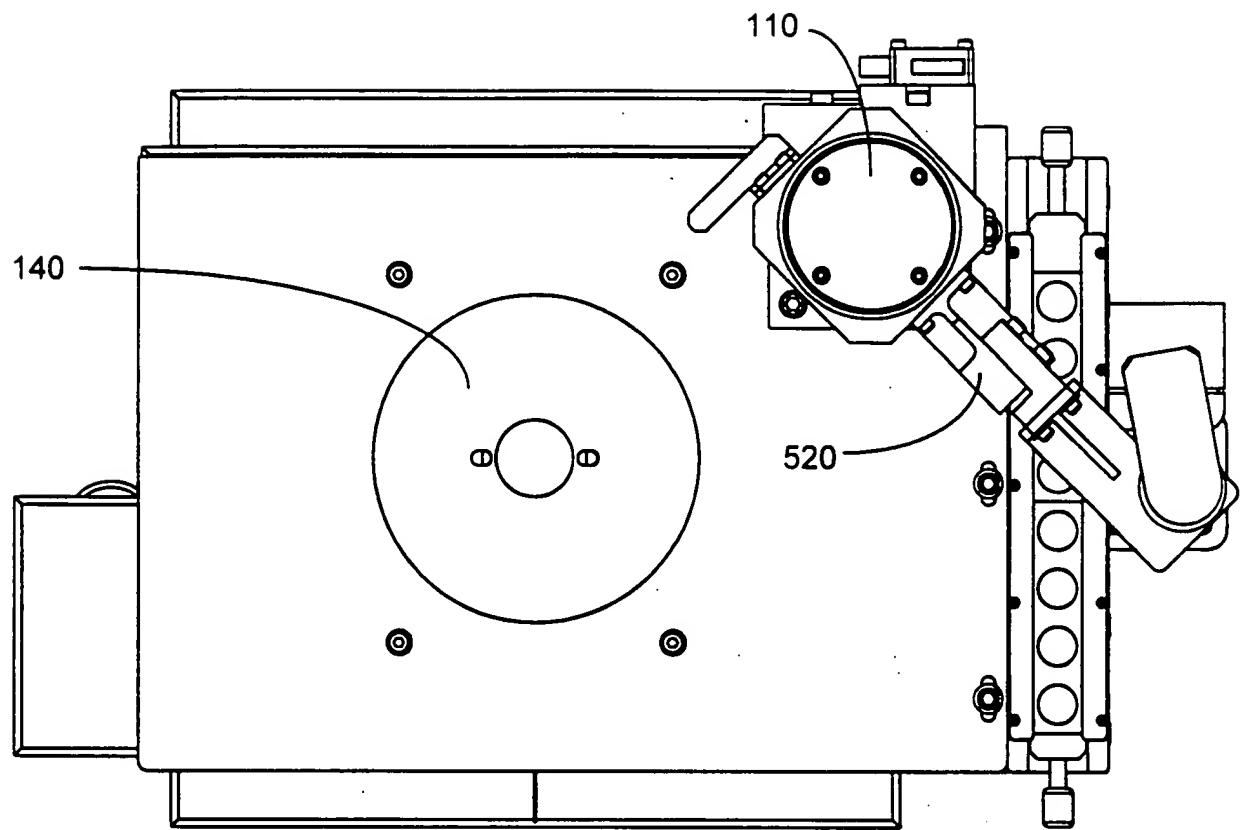


FIG. 11

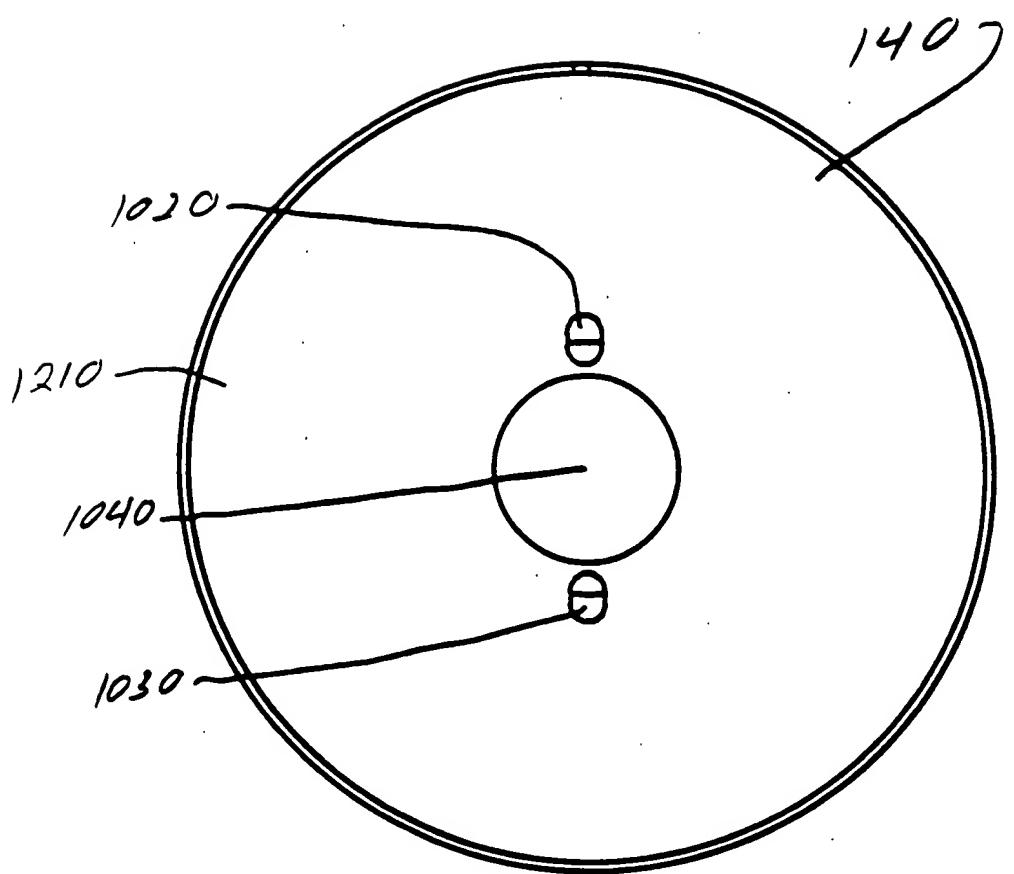
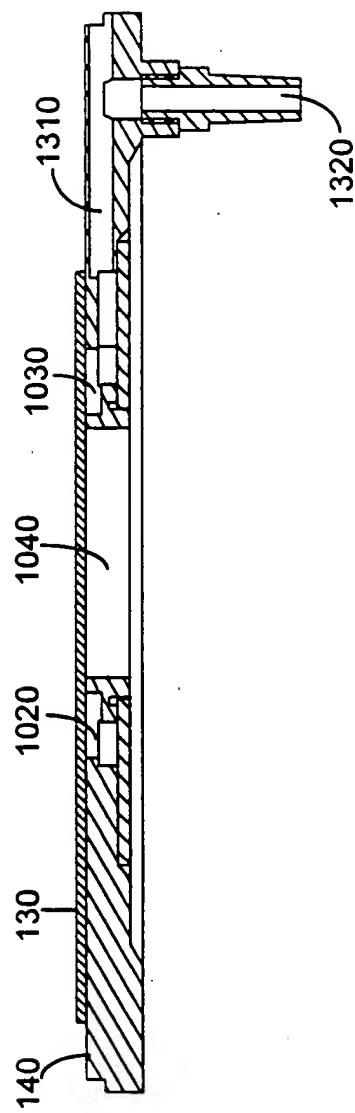


FIG. 12

FIG. 13



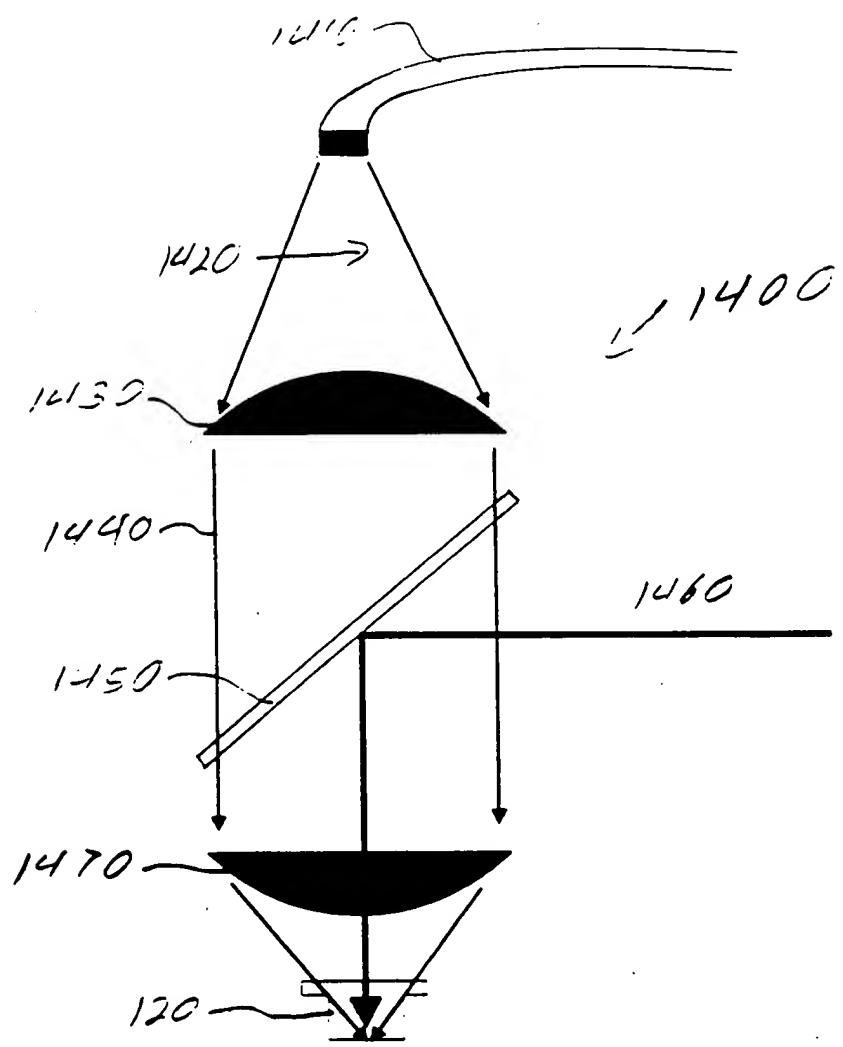


FIG 14

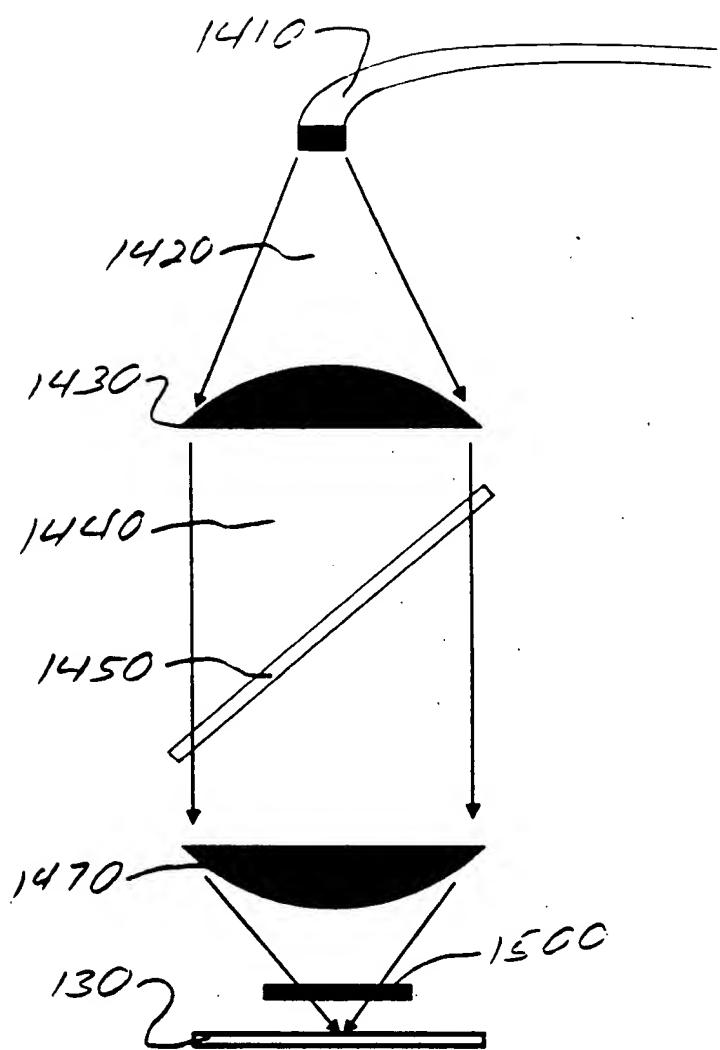


FIG 15

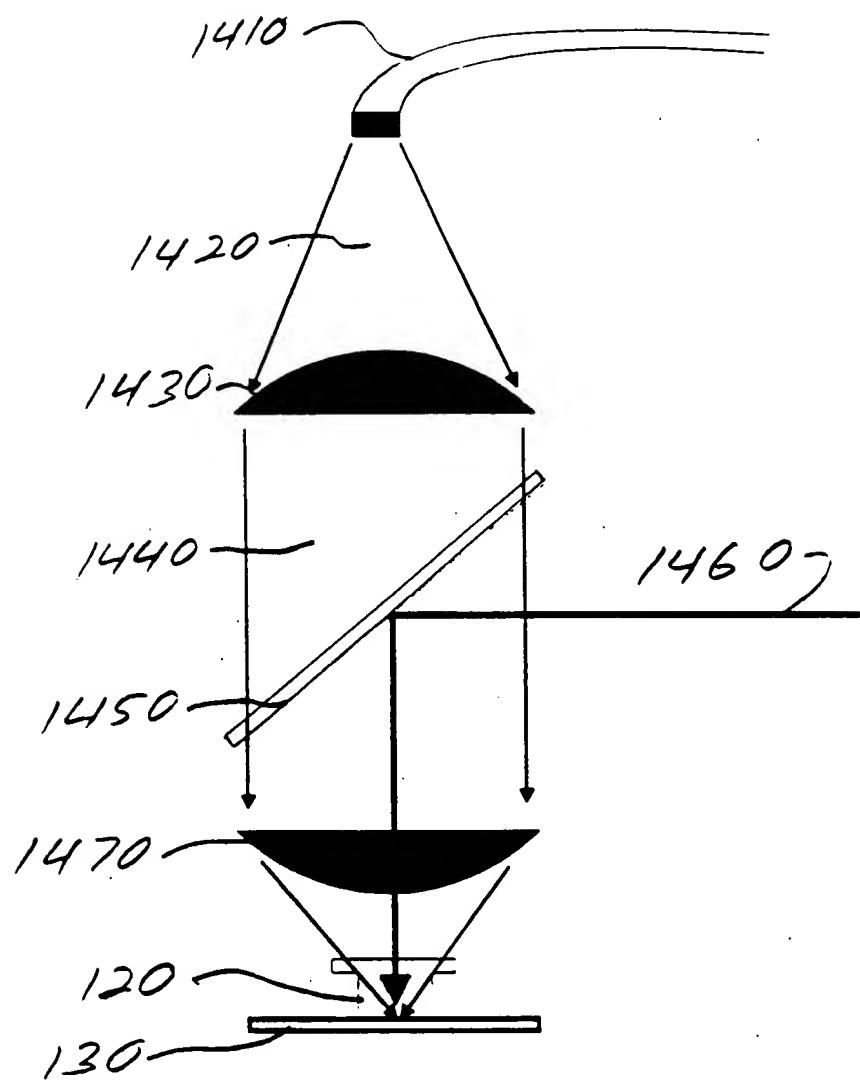


FIG. 16

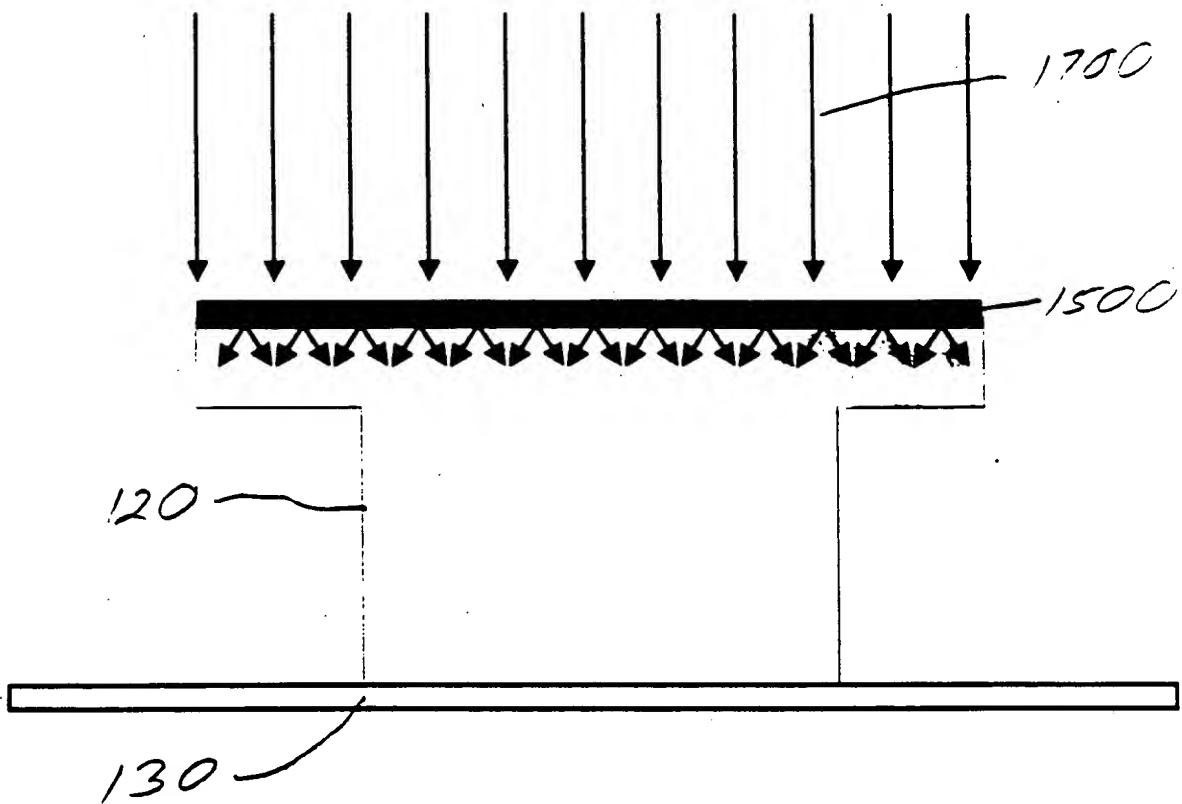
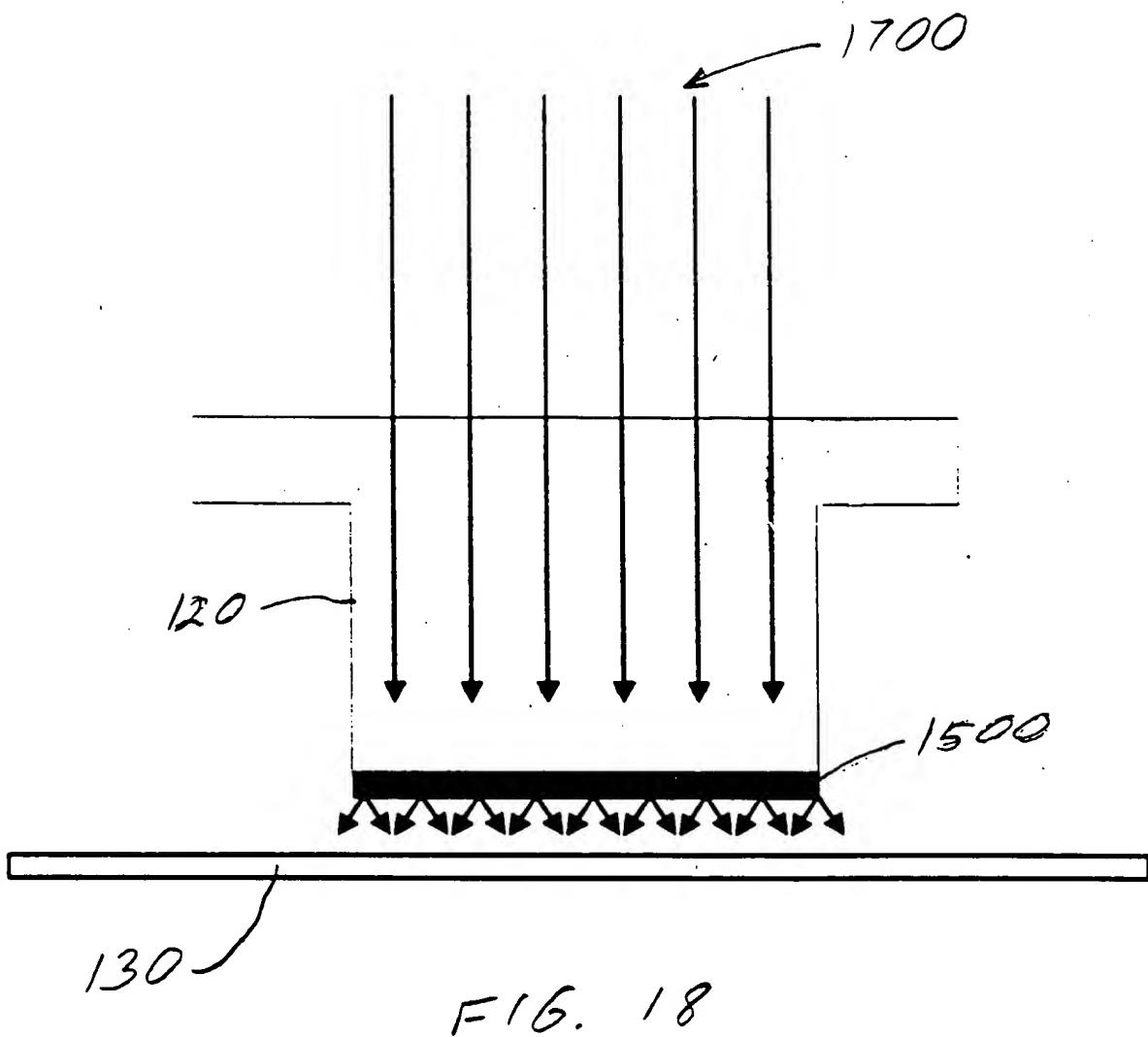


FIG. 17



PATENT
Attorney Docket No. 17726-709

CONSUMABLE FOR LASER CAPTURE MICRODISSECTION

Inventors: Thomas M. Baer; David F. Head; and John Toeppen

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part under 35 U.S.C. 120 of copending U.S. Ser. No. 60/060,732, filed October 1, 1997, now pending, the entire contents of which are hereby incorporated by reference as if fully set forth herein.

5

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the field of laser capture microdissection (LCM). More particularly, the present invention relates to apparatus for acquiring LCM samples that include an LCM film mounted on at least a part of the interior of an analysis container. Specifically, a preferred implementation of the present invention relates to a substantially planarized ethylene vinyl acetate (EVA) polymer LCM film that is hot vacuum baked onto the bottom of a microcentrifuge tube cap. The present invention thus relates to an LCM sample acquisition apparatus of the type that can be termed planar cap.

15 2. Discussion of the Related Art

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to 20 mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection 25 (LCM). Laser capture microdissection is a one-step technique which integrates a standard

laboratory microscope with a low-energy laser and a transparent ethylene vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, *in situ* breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research. For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP,

laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, 5 published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is 10 placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. 15 This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

20 Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90°C.

The thermoplastic EVA films used in LCM techniques have been doped with dyes, 25 such as an infrared napthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye 30 concentration in the plastic is about 0.001 M.

While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

5 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

10

SUMMARY OF THE INVENTION

An object of the invention is to improve the speed of the laser capture microdissection technique. Another object of the invention is to improve the accuracy of the laser capture microdissection technique. Another object of the invention is to improve the reproducibility 15 of the laser capture microdissection technique. Yet another object of the invention is to reduce the amount of contamination involved with the laser capture microdissection technique. Therefore, there is a particular need for an LCM consumable that integrates an LCM film into the interior of an analysis container. A planar cap includes a substantially planarized ethylene vinyl acetate (EVA) polymer LCM film that is hot vacuum baked onto 20 the bottom of a microcentrifuge tube cap. The laser capture microdissection caps can be shipped as-baked (i.e., packaged without post-bake processing) to protect the laser capture microdissection transfer film and minimize contamination. The cap, and the configuration in which it is shipped, provides the additional advantages of quick and easy utilization. Thus, it is rendered possible to simultaneously satisfy the requirements of speed, accuracy and 25 resistance to contamination, which, in the case of the prior art, are mutually contradicting and cannot be simultaneously satisfied.

A first aspect of the invention includes a laser capture microdissection assembly comprising: a plate having a substantially planar top surface; and at least one laser capture microdissection cap connected to said substantially planar top surface of said plate, wherein 30 said at least one laser capture microdissection cap includes a transfer film carrier having a substrate surface; and a substantially planarized laser capture microdissection transfer film

connected to said substrate surface of said transfer film carrier. A second aspect of the invention includes a laser capture microdissection apparatus, comprising: a transfer film carrier having a substrate surface; and a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier, said laser capture microdissection transfer film including at least one integrally formed structural feature that protrudes and provides a controllable spacing between said laser capture microdissection transfer film and a sample. A third aspect of the invention includes an integral portion of a biological reaction vessel, comprising: a transfer film carrier having a substrate surface; and a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier. A fourth aspect of the invention includes a laser capture microdissection assembly comprising: a plate having a top surface; and at least one laser capture microdissection cap coupled to said top surface of said plate, wherein each of said at least one laser capture microdissection cap includes a transfer film carrier having a substrate surface; and a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier.

A fifth aspect of the invention includes a method of making the laser capture microdissection assembly comprising: providing a plate having a substantially planar top surface; providing at least one laser capture microdissection cap, said at least one laser capture microdissection cap including a transfer film carrier having a substrate surface; providing a laser capture microdissection transfer film adjacent to said substrate surface of said transfer film carrier; and hot vacuum baking said at least one laser capture microdissection cap and said plate so as to substantially planarize said laser capture microdissection transfer film. A sixth aspect of the invention includes a method of making a laser capture microdissection consumable, comprising: providing a transfer film carrier having a substrate surface; and forming a laser capture microdissection transfer film on said substrate surface, wherein forming includes hot vacuum baking said laser capture microdissection transfer film. A seventh aspect of the invention includes a method of making an integral portion of a biological reaction vessel, comprising: providing a transfer film carrier having a substrate surface; and fabricating a laser capture microdissection transfer film on said substrate surface. An

- eight aspect of the invention includes a method of making a laser capture microdissection assembly, comprising: providing a plate having a top surface; providing at least one laser capture microdissection cap, said at least one laser capture microdissection cap including a transfer film carrier having a substrate surface;
- 5 providing, for said at least one laser capture microdissection cap, a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier; placing said at least one laser capture microdissection cap in contact with said plate; and hot vacuum baking both said at least one laser capture microdissection cap and said plate so as to produce said laser capture microdissection assembly.
- 10 A ninth aspect of the invention includes a method of imaging a sample with a microscope, comprising: providing said microscope; locating a scattering media within a beam path defined by said microscope and within a few millimeters of a sample; and imaging said sample through said scattering media with said microscope. A tenth aspect of the invention includes a microscope, comprising: a scattering media
- 15 located within a beam path defined by said microscope and within a few millimeters of a sample.

These, and other, aspects of the present invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following

20 description, while indicating preferred embodiments of the present invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the present invention without departing from the spirit thereof, and the invention includes all such modifications.

25

BRIEF DESCRIPTION OF THE DRAWINGS

A clear conception of the advantages and features constituting the present invention, and of the components and operation of model systems provided with the

present invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be
5 given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

Figs. 1A-1C illustrate three views of a laser capture microdissection (LCM) sample plate, representing an embodiment of the present invention;

10 Figs. 2A-2C illustrate three views of the sample plate shown in FIGS. 1A-1C after coating with a release agent, representing an embodiment of the present invention;

Figs. 3A-3D illustrate four views of a sample carrier, representing an embodiment of the present invention;

15 Figs. 4A-4D illustrate four views of the sample carrier illustrated in FIGS. 3A-3D after an LCM film is added, representing an embodiment of the present invention;

Figs. 5A-5C illustrate three views of an assembly that includes four of the sample carriers depicted in FIGS. 4A-4D and one of the plates depicted in FIGS. 2A-2C, representing an embodiment of the present invention;

20 Figs. 6A-6C illustrate three views of a completed assembly after vacuum hot cast molding, representing an embodiment of the present invention;

Figs. 7A-7B illustrate two sequential views of a laser capture microdissection film with molded features, representing an embodiment of the present invention;

Fig. 8 illustrates a bottom view of a laser capture microdissection film with
25 molded features, representing an embodiment of the present invention;

Fig. 9 illustrates a side view of a laser capture microdissection apparatus,
representing an embodiment of the invention;

Fig. 10 illustrates a side view of a microcentrifuge tube cap with a negative draft, representing an embodiment of the invention; and

Figs. 11A-11D illustrates a several views of a biological reaction vessel, representing an embodiment of the invention.

5

DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the present invention in detail.

The entire contents of U.S. Ser. No. 60/unknown, filed February 7, 1997 (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed October 1, 1997; and U.S. Ser. No. 15 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

Turning to Figs. 1A-1C, a plate 100 is depicted. Plate 100 can be fabricated from metal, glass, ceramic, or any other material suitable for the subsequent processing steps described below. In a preferred embodiment, plate 100 is a glass microscope slide. It is important that the top surface 101 of plate 100 be flat. Although the depicted embodiment shows a bare microscope slide, the plate can be coated, or otherwise surface treated, in a preliminary processing step.

Turning now to Figs. 2A-2C, the plate 100 is depicted with a release agent 210. The release agent 210 is applied to the top surface 101. It will be noted that the top surface 101 is obscured by the release agent 210 in Figs. 2A-2B but is clearly visible as an interface in Fig. 2C.

The release agent can be any suitable nonadhesive material such as, for example, silicones, or TEFLON (i.e., polytetrafluoroethylene). Advantageously, the release coating can be a surfactant that increases the contact angle of liquids with which it comes in contact. 30 It is important that the release agent 210 maintain and extend the flatness provided initially by

the top surface 101. In a preferred embodiment, the release agent 210 can include a silicone containing surfactant agent such as, for example, RAIN-X.

Turning now to Figs. 3A-3D, a sample carrier 300 is depicted. The sample carrier 300 has an upper portion 310 and a lower portion 320. The upper portion 310 includes a top 5 surface 315 and an outer perimeter 317, and a shoulder 319. The lower portion 320 includes a flare 322, an inner perimeter 324, a taper 326 and a substrate surface 328.

The sample carrier 300 can be a polymeric cap that is of transparent optical quality. For example, the cap could be fabricated from polycarbonate, or other suitable optically 10 transparent plastic. However, the cap does not need to be optically transparent provided the absorption characteristics of the polymer from which it is made are compatible with suitable transmission of the laser energy to the capture film.

Turning now to Figs. 4A-4D, a laser capture microdissection (LCM) transfer film 400 is shown being applied to the sample carrier 300. It will be appreciated that the LCM transfer 15 film 400 is depicted out of scale for the sake of clarity. The laser capture microdissection transfer film 400 can be applied to the bottom of a circular cap by punching a circular section from a free-standing sheet of ethylene vinyl acetate. Alternatively, the LCM transfer film 400 can be molded to the bottom of the cap. The LCM transfer film 400 can be deposited on the cap using a process such as spin coating, dipping, or spraying. In any event, manufacture of the consumable should be carried out in a sterile environment.

20 It is advantageous that the LCM transfer film 400 be thin. For example, a 50 micron thick film is preferable to a 100 micron thick film. However, the film can advantageously be fabricated in thicknesses of approximately 500, 400, 300, 200, 100, 50 microns, or less.

Turning to Figs. 5A-5C, a plurality of combined sample carriers 300 together with their corresponding LCM transfer films 400 are depicted being lowered toward the release 25 agent 210 that is coated on top of the plate 100. The LCM transfer films 400 can be an ethylene vinyl acetate (EVA) polymeric material. It will appreciated that Fig. 5A depicts the assembly process at an earlier point in time compared to Fig. 5C wherein the gap between the LCM transfer film 400 and the release agent 210 is almost closed.

30 Turning now to Figs. 6A-6C, the assembly of four sample carriers 300 on plate 100 is depicted during the process step of vacuum hot baking. The process of vacuum hot baking causes the EVA to soften, melt and flow thereby conforming to the substantially planar

surface presented by the release agent 210. In this way, the flatness possessed by the plate 100 is transferred to the LCM transfer film 400. This also eliminates trapped air.

The hot vacuum baking of the film can take place in moderate vacuum. In a preferred embodiment, the hot cast molding takes place at one torr and 95 degrees C for approximately 5 one hour.

In an alternative embodiment, instead of attaching the LCM film to the base of the cap prior to its placement on top of the release agent coated plate, the LCM film can be coated on top of the release agent as a film layer. A sample carrier can then be placed on top of the LCM film. An assembly of one, or more, such combinations can then be subjected to hot 10 vacuum melt casting to planarize at least that portion of the LCM film that is located at the interface between the sample carrier and the release agent. In this way, when the sample carrier is removed from the plate, a portion of the planarized LCM film that corresponds with the bottom surface of the sample carrier will be broken away from the assembly together with the cap that is being removed. Those portions of the LCM film that are not adjacent the 15 bottom of the cap being removed will remain on the plate. In a preferred embodiment, when the sample carrier is pulled away from the plate, a twisting motion is applied to the sample carrier either before and/or during linear separation of the two prime components so as to exert a sheer force both within the LCM film and between the LCM film and the release layer.

20 The release coating can be a silicone. Alternatively, the release coating can be a polytetrafluoroethylene.

Throughout this specification, the more descriptive phrase "transfer film carrier" can be substituted for the phrase "sample carrier." In general, the transfer film carrier carries the transfer film. Only that portion of the sample that is transferred to the transfer film is carried 25 by the carrier.

The ethylene vinyl acetate can be selected from among the available materials based on the following criteria. The ethylene vinyl acetate should have a high melt index. A high melt index is indicated by low viscosity and low molecular weight.

It is important that the ethylene vinyl acetate, or other material being used for the 30 LCM transfer film, have a modest tack. Thus, the transfer film is somewhat sticky but will not bind to everything with which it comes in contact.

The caps can be made from clear plexiglass G (i.e., polymethyl methacrylate). By treating the glass slide with a surfactant before the caps are vacuum hot cast in place, the completed caps can be popped off the glass slide just before they are needed for acquisition of sample material.

- 5 In a preferred embodiment, the cap is sized to fit in a standard microcentrifuge tube. The LCM transfer film can be attached to the cap using glue, or by welding the thermoplastic, or by some other mechanical means, holding the film in place.

The side walls of the cap can have a negative draft. This negative draft can be machined into the tooling with which the caps are made.

- 10 After capturing the tissue to be analyzed on the bottom of the cap, the cap is placed on the microcentrifuge tube containing proteinase (i.e., protease, e.g., Trypsin) solution and the tube is inverted. The tissue is then dissolved and the DNA is free to enter the solution. The solution is then pipetted out of the tube and into the PCR mixture.

While not being bound by theory, it is believed that the EVA film expands both up
15 and down when it is exposed to the energy from the laser. As an approximation, it is believed that the EVA film expands approximately 12-15% downward and upward when it is exposed to the LCM charge from the laser. The upward expansion is restricted by the plastic cap.

- The thickness of the LCM transfer film should be held to within 20%, preferably 5%.
The bottom, exposed surface of the LCM transfer film can be termed a capture surface. The
20 flatness of the LCM transfer film should be held to within approximately five microns, preferably approximately one micron. The flatness of the film can readily characterized based on the number of fringes multiplied by $\lambda/2$. The flatness of the LCM transfer film should preferably be held to within two waves which is approximately equal to 1/4 micron per fringe, given a λ of 540nm.

25 The dye in the ethylene vinyl acetate is what absorbs the laser energy. The ethylene vinyl acetate transforms to a liquid phase, infuses into the cell structure of interest and then hardens.

- The particular manufacturing process used for fabricating the assembly should be inexpensive and reproducible. Conveniently, the fabrication of the present invention can be
30 carried out by using any coating and baking method. It is preferred that the process be

conducted in a contaminant-free environment. For the manufacturing operation, it is moreover an advantage to employ an automated method.

However, the particular manufacturing process used for fabricating the assembly is not essential to the present invention as long as it provides the described assembly. Normally 5 those who make or use the invention will select the manufacturing process based upon tooling and energy requirements, the expected application requirements of the final product, and the demands of the overall manufacturing process.

The particular material used for the cap should be biologically and chemically inert. Conveniently, the cap of the present invention can be made of any material with a melting 10 point higher than that of EVA. It is preferred that the material be inexpensive. For the manufacturing operation, it is moreover an advantage to employ a transparent thermoplastic material that can be injection molded or machined. For example, the cap can include polymethyl methacrylate. By proper selection of the polymeric materials, the cap can be solid. There is no need for a through-hole through the center axis of the cap.

15 However, the particular material selected for the cap is not essential to the present invention, as long as it provides the described function. Normally, those who make or use the invention will select the best commercially available material based upon the economics of cost and availability, the expected application requirements of the final product, and the demands of the overall manufacturing process.

20 The LCM transfer film can be any suitable thermoplastic. For example, the LCM transfer film can include one or more of: EVAs; polyurethanes (PU); polyvinyl acetates; ethylene-methyl acrylate (EMAC); polycarbonate (PC); ethylene-vinyl alcohol copolymers (EVOH); polypropylene (PP); and expandable or general purpose polystyrene (PS). ELVAX 410, 200 and 205 are suitable resins of EVA that are commercially available from DuPont 25 wherein the operative variant is the amount of vinyl.

The LCM transfer film can include an absorptive substance. The absorptive substance can include an absorptive dye. This dye can be either a broad band absorptive dye or a frequency specific absorptive dye. For example, the absorptive dyes can include one or more of: tin(IV) 2,3-naphthalocyanine dichloride; silicon(IV) 2,3-naphthalocyanine dihydroxide; 30 silicon (IV) 2,3-naphthalocyanine dioctyloxide; and vanadyl 2,11,20,29-tetra-tert-butyl-2,3-

naphthalocyanine. Also, the absorptive substance can include a plurality of Fullerenes (i.e., Bucky Balls, e.g., C₆₀).

The LCM transfer film can also include a scattering media. Since the LCM transfer film is very close to the sample, the scattering media reduces shadows, thereby improving the process of imaging. The scattering media can include a diffusing material. For example, the LCM transfer film can be loaded with a small particulate material that scatters the illumination light so as to minimize shadows and improve imaging without detrimentally effecting the LCM beam. Alternatively, the transfer film can include a dichromatic gelatin (DCG) to perform the same functions. The DCG can be exposed and developed to provide specific diffuser properties within the transfer film such as shaping.

There are a variety of techniques for building a noncontact LCM transfer film and/or carrier. The purpose of the noncontact LCM approach is to provide a method for the elimination of problems associated with nonspecific binding of tissue to an LCM film. In more detail, if a sample slide has areas with loosely attached cells, these portions of the sample can be lifted mistakenly from the slide due to nonspecific attachment to the LCM film. That is, these areas stick to the film even though they were not illuminated by the laser. If these portions are transferred to the reagent vessel they will be digested by the reagents and appear as contaminants in the sample. It is important to prevent the loosely bound tissue areas from contacting the film.

One method for preventing the contact of the film to areas of tissue that might nonspecifically transfer is to offset (distance) the film a few microns from the tissue sample. In the area illuminated by the laser, the film expands roughly 10% of its thickness (about 5 to 10 microns based on a typical thickness of 50 to 100 microns) and contacts the tissue, thereby allowing transfer in the illuminated region. Outside this region, the film and tissue never come in contact because the film is spaced away from the tissue. The film, however, must not be spaced too far from the tissue (greater than a few microns) since the film needs to contact the tissue after it expands due to the laser illumination.

One technique to make a noncontact LCM transfer film that "stands-off" a few microns is to create a series of pedestals that are a few microns high so as to provide a series of standoffs for the cap to rest on. These pedestals can be created by exposing edges of the transfer film to the focused laser beam. The laser beam distorts the normally flat film in the

focal region raising the surface in this region. By placing these pedestals at the vertices of an equilateral triangle with points located at the rim of the transfer film carrier a good three-point mount is provided. The height of these pedestals can be adjusted by changing the power and pulse length of the focused laser beam. The diameter can be adjusted by changing
5 the diameter of the laser beam. The exposure levels are similar to the levels used for tissue transfer: approximately 10-90 mW for approximately 10-90 milliseconds. (To create the pedestals it may help to expose the film when it is in contact with a glass slide.) The reagent vial can be constructed so that it has an internal rim that contacts the pedestals, sealing them from the reagent, thereby preventing tissue that might be on the pedestals from contaminating
10 the sample.

Turning now to Figs. 7A-7B, an LCM film 700 can be provided with features 710. The features 710 can include a raised portion 720 (pedestal) and a protruding feature 730 (e.g., rim). The features 710 can be molded (e.g., replicated), or otherwise formed (e.g., by laser), in the LCM film 700. Such features give the LCM film 700 a working surface that
15 defines a topography.

The purpose of the features 710 is to provide an additional way of selecting single cells from a tissue sample using LCM, other than just a very small laser spot size. The features 710 that are fabricated into the LCM transfer film can be roughly the size of a desired cell 740. The features 710 can extend out from the film surface for a distance of several
20 microns.

The film 700 itself can be offset from the cells a distance of from approximately 5 to approximately 10 microns by the protruding feature 730 that runs around the circumference of the cap. To stabilize the plane of the film, it will be appreciated that the protruding feature only needs to extend along at least three points of a perimeter of the film and does not need to
25 be a continuous rim.

The features 710 can be fabricated by hot cast molding the LCM film 700 against a mold that has complimentary shapes of the features laser machined into the mold surface. Such a mold can be made out of a polished metal surface or a glass surface using a Q-switched laser focused to a diameter of from approximately 5 to approximately 20 microns.
30 The features 710 can also be fabricated by molding the film against a mold surface that is

micromachined with a diamond stylus. The topography is transferred from the mold to the film via replication.

A protuberance (raised portion 720) for acquiring the desired cell 740 can include a small raised area of LCM film roughly 5 to 20 microns in diameter. When a laser beam 750 5 heats this portion of the film, the raised portion 720 will contact the tissue first and the laser power can be adjusted so that the surrounding adjacent film regions do not contact the tissue. Thus, the raised portion 720 provides spatial discrimination in addition to the spatial 10 discrimination provided by the position, size and mode of the laser beam. An advantage of the features 710 is that a larger laser beam could be used and a researcher or laboratory technician could still achieve single cell lift-off. The raised portion of the film (raised portion 15 720) will be heated to a higher temperature than the surrounding flat film area. The protruding feature 730 (i.e., the rim) will not be heated. This would also increase the likelihood that a cell in the region of the feature would be captured exclusively. Of course, it is advantageous that raised portion 720 not protrude as far as protruding feature 730.

Referring now to Fig. 8, multiple pedestals 800 could be molded into an LCM film 810 to allow multiple single cell lift off regions. The LCM film 810 could again include a rim 820. Multiple cells could then be analyzed in a single microcentrifuge tube.

The structural feature (i.e., spacer) that holds the film away from the sample can be hot vacuum baked into the transfer film. According to this process, a negative of the 20 structural feature can be formed in a plate. The structural feature is then replicated (as a positive) in the film when it is heated and flows into the void defined by the negative of the feature. Alternatively, the structural feature can be formed in the transfer film with the use of a laser, or even with micro-machining equipment.

The structural feature, or spacer, can be integrally formed in the laser capture 25 microdissection transfer film. The structural feature provides a separation between the transfer film and the sample. This separation holds the film away from the sample, thereby enabling noncontact laser capture microdissection.

The transfer film can be connected to the substrate surface with a refractive index matching transparent fluid or glue. Alternatively, the transfer film can be coupled to the 30 substrate surface by punching both the sample carrier and the transfer film from stock

material simultaneously. It is even possible to couple the film to the carrier with double-sided tape.

The laser capture microdissection transfer film includes a substantially planarized low land area. This low land area can be provided with structural features that protrude so as to define a laser capture microdissection acquisition zone. These protrusions can be termed pedestals. The low land can also be provided with structural features that hold most of the film away from the sample. In order to support the plane of the film, it is preferable to have at least three such supporting features. If these supporting features run around most, or all, of a perimeter of a transfer film, they can be termed a rim.

Whatever contacts the tissue needs to be equidistant from the tissue so that the dosimetry is constant across the transfer film. In this way, a known distance between the tissue and the transfer film can be established. In many cases such a known distance will be fixed across substantial portions of the transfer film surface. However, it is sufficient that the distance be known and does not need to be fixed. The distance needs to be known for the purpose of adjusting laser power so as to achieve tissue transfer.

When the transfer film is exposed to the electromagnetic energy, it expands (both up and down) against the substrate surface and contacts the tissue, thereby injecting itself into the sample. In the case where there is a space between the transfer film and the top surface of the sample, (noncontact laser capture microdissection) the expanding film will be projected through that space before it contacts the top surface of the sample at the beginning of the injection phase.

Referring now to Fig. 9, a scatter illuminator design for an LCM device is illustrated. The purpose of the scatter illuminator design is to provide a more appropriate illuminator for an LCM microscope that generates a more even illumination to prevent shadows from obscuring internal cell structure.

A laser capture microdissection apparatus includes a top portion 910 and a bottom portion 920. The top portion 910 includes an upper surface to which a scattering media 930 can be coupled. The bottom portion 920 includes a substrate surface to which a scattering media 940 can be coupled. Either, or both, of the scattering media 930 and 940 can be used. The scattering media can be incorporated into the transfer film carrier and/or the LCM transfer film.

Using a standard inverted microscope light source and placing a scattering media (e.g., a piece of paper) near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type eliminates the need for refractive index matching of the sample. Such a scattering media can 5 allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk glass which is a very dense, fine diffuser available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be 10 used as the scattering media. Other types of transparent scattering media can be used, such as, for example, frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser. In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse 15 the illumination light sufficiently.

The scattering media can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The 20 scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

Referring now to Fig. 10, a laser capture microdissection apparatus 1000 is illustrated. The apparatus 1000 includes a top portion 1010 and a bottom portion 1020. The bottom portion 1020 includes a negative draft 1030. The negative draft 1030 is preferably 25 approximately 5°. The bottom portion 1020 also includes a chamfer 1040. The chamfer 1040 is preferably approximately 20°. The bottom portion 1020 also includes a girdle 1050. The width of the girdle 1050 for line contact with the interior of an analysis vessel is preferably approximately 0.01". Caps with a negative draft can be fabricated with a break-apart plastic injection molding die. Alternatively, negative draft caps can be fabricated by 30 interpolation with computer numeric control cutting tool machinery.

Turning now to Figs. 11A-11D, a laser capture microdissection (LCM) biological reaction vessel 1100 including an analysis vessel 1110 with an internal ridge and a cap 1120 with a transfer film 1130. The transfer film 1130 can include EVA and can have a stand-off rim 1150. Stand-off rim 1150 can be a 10-20 micron ridge providing a noncontact region in 5 the center of the transfer film 1130. The cap 1120 is an integral portion of the biological reaction vessel 1100. The analysis vessel 1110 is formed to include an internal ridge 1140. The internal ridge slopes back toward an opening in the analysis vessel 1110 so as to make a tight seal with the cap 1120, even if the stand-off rim is not present. The purpose of combining the internal ridge 1140 with the stand-off rim 1150 in a single embodiment is to 10 provide an LCM analysis vessel and film carrier that have features to facilitate a noncontact method for positioning the transfer film over the tissue sample. The LCM non-contact method reduces the probability that areas of tissue outside the focal adhesion region will be transferred. However, if the stand-off rim 1150 later comes in contact with the reaction, this advantage will be lost. The analysis vessel 1110 with this internal sealing feature allows the 15 transfer film 1130, with stand-off rim 1150, to contact the tissue but not contact reaction fluid in the analysis vessel 1110.

The biological reaction vessel 1100 includes the cap 1120 (lid) that can be removably coupled to the analysis vessel 1110. The transfer film 1130 is attached to the clear plastic cap 1120. The transfer film 1130 can be hot cast molded to include the stand-off rim 1150 that is 20 10 microns thicker than the central region of the cap 1120. The stand-off rim 1150 can be termed an annular rim. The transfer film 1130 expands in the region of the focused laser beam and is able to bridge the 10 micron gap, thereby contacting the tissue and allowing transfer of a portion of the tissue to the film. This stand-off rim 1150 can be termed a standoff region and acts as a spacer elevating the central region of the transfer film 1130 25 above the tissue and preventing the transfer film 1130 from contacting the tissue in this central region, until the LCM laser activate the transfer film 1130. This stand-off region feature can be molded into the transfer film 1130 by pressing the transfer film 1130 onto a heated plate that contains an inverse image of this step (spacer) feature. This method replicates the feature. Such a mold could be constructed using a polished metal plate and 30 standard chemical etching techniques. It could also be manufactured using glass or silicon

substrates and chemical etching. Alternatively, a diamond lathe could be used to machine this feature onto a suitable metal substrate (e.g., copper, aluminum, steel, etc.).

The cap 1120 that seals the liquid reagent analysis vessel 1110 can be made out of inert plastic such as polypropylene or polyethylene. The analysis vessel 1110 has the internal ridge 1140 (step) that is designed to mate with and cover the annular rim of the cap 1120 providing a tight seal at this point. This seal prevents liquids in the analysis vessel 1110 from contacting the bottom surface of the rim of the cap. This design eliminates nonspecific tissue transfer since the stand-off rim 1150 is the only area of the cap 1120 that contacts the tissue (other than the desired transfer regions illuminated by the laser) and the digestion reagents in the analysis vessel 1110 never contact this region (stand-off rim 1150). The internal ridge 1140 feature in the analysis vessel can be designed with a slight angle so as to partially cut into the Transfer film 1130 providing a very tight seal similar to vacuum flange sealing techniques. A slight bulge or indentation can be molded into the barrel of the cap 1120 or into the top portion of the analysis vessel 1110 so as to provide a downward directed force and a positive seal between the cap 1120 and the analysis vessel 1110.

Example

A specific embodiment of the present invention will now be further described by the following, nonlimiting example which will serve to illustrate in some detail various features of significance. The example is intended merely to facilitate an understanding of ways in which the present invention may be practiced and to further enable those of skill in the art to practice the present invention. Accordingly, the example should not be construed as limiting the scope of the present invention.

In an exemplary embodiment of the invention, a glass microscope slide is first cleaned. Then the glass microscope slide is spray coated with a thin layer of a commercially available silicone release agent, in this example a silicone containing surfactant that is readily commercially available (i.e., RAINEX). Meanwhile, a supply of sample carriers in the form of microcentrifuge tube caps are molded from plexiglass G. Cylindrical chips of LCM film punched from a sheet of ethylene vinyl acetate (EVA) are then attached to the bottom surface of the caps, optionally with an epoxy adhesive. The resultant cap subassemblies are then placed on top of the release agent coated glass subassembly for hot vacuum baking. The hot vacuum baking is carried out at a pressure of approximately one torr or less at a temperature

of 95°C for approximately one hour. This planarizes the transfer film. The baked assembly is then allowed to cool to room temperature. The resulting assembly can include a plano-concave void located between each of the caps and the underlying plate. In this way only the perimeter of the bottom of the caps is in contact with the glass plate. This provides two significant advantages. First, the working surface of the LCM film is spaced apart from the glass slide in a vacuum and remains free of surface damage and contaminants. Second, the removal of each cap from the glass slide is facilitated by the fact that only a fraction of the surface area of the bottom of the cap is attached to the release layer that has been coated on the glass slide. Therefore, removal of the cap from the slide requires much less force than if the entire lower surface of the cap were in contact with the release layer.

It can be appreciated that by both making and shipping the cap on the same glass slide, the number of processing and packaging steps is reduced while reproducibility and cleanliness are improved.

The completed consumable products can be sterilized (e.g., with beta or gamma radiation). Finally, the completed consumable products should be subjected to a rigorous quality assurance inspection.

There are a number of advantages to leaving the caps on the slide until they are about to be used. These advantages include protection of the optically flat surface. For example, leaving the caps on the slide reduces hydroxyl contamination of the transfer film. These advantages also include the prevention of particulate matter from settling on the surface.

Practical Applications of the Invention

A practical application of the present invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases. This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The present invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The present invention will allow medical doctors to compare actual patient tissue samples with archived data from patient samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the present invention will find use are drug discovery, developmental biology, forensics, botany, and the study of

infectious diseases such as drug-resistant tuberculosis. There are virtually innumerable uses for the present invention, all of which need not be detailed here.

Advantages of the Invention

- Laser capture microdissection, representing an embodiment of the invention can be
- 5 cost effective and advantageous for at least the following reasons. The present invention will replace current methods with better technology that allows for more accurate and reproducible results. The present invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection film into the interior surface of an analysis container such as a microcentrifuge tube.
- 10 All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the present invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the present invention may be made without deviating from
- 15 the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

- For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and
- 20 assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the caps and cap assemblies disclosed herein are described as a physically separate module, it will be manifest that the caps and cap assemblies may be integrated into other apparatus with which they are associated. Furthermore, all the disclosed
- 25 elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

- It is intended that the appended claims cover all such additions, modifications and rearrangements. The claims are not to be construed as including means-plus-function
- 30 limitations, unless such limitations are explicitly recited using the term "means" in the claims.

Expedient embodiments of the present invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection apparatus, comprising:
a transfer film carrier having a substrate surface; and
a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier, said laser capture microdissection transfer film including at least one integrally formed structural feature that protrudes and provides a controllable spacing between said laser capture microdissection transfer film and a sample.
2. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film includes a material, that upon exposure to sufficient electromagnetic energy, expands and projects itself away from said substrate surface.
3. The laser capture microdissection apparatus of claim 1, further comprising a scattering media in proximity to said laser capture microdissection transfer film.
4. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film includes an absorptive substance.
5. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film is hot vacuum baked onto said substrate surface.
6. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film is bonded to said substrate surface with a refractive index matching transparent glue.
7. The laser capture microdissection apparatus of claim 1, wherein said transfer film carrier includes a negative draft such that a distal diameter defined by said surface of said transfer film carrier is greater than a proximal diameter defined by an inner perimeter of said transfer film carrier.

8. The laser capture microdissection apparatus of claim 7, wherein said transfer film carrier includes a girdle that is contiguous with said negative draft.
9. The laser capture microdissection apparatus of claim 7, wherein said transfer film carrier includes a chamfer that is contiguous with said substrate surface.
10. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film has a thickness that is less than 500 microns.
11. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film has a thickness that is held to within 20%.
12. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film has a capture surface that is opposite said substrate surface, said capture surface having a flatness that is held within five microns.
13. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film includes at least one pedestal that protrudes and defines a laser capture microdissection acquisition zone.
14. The laser capture microdissection apparatus of claim 1, wherein said laser capture microdissection transfer film includes a protruding feature that runs along at least three points of a perimeter of said laser capture microdissection transfer film.
15. A microcentrifuge tube cap comprising the laser capture microdissection apparatus of claim 1.
16. An integral portion of a biological reaction vessel, comprising:
 - a transfer film carrier having a substrate surface; and
 - a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier.

17. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film includes a material, that upon exposure to sufficient electromagnetic energy, expands and projects itself away from said substrate surface.
18. The integral portion of a biological reaction vessel according to claim 16, further comprising a scattering media in proximity to said laser capture microdissection transfer film.
19. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film includes an absorptive substance.
20. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film is hot vacuum baked onto said substrate surface.
21. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film is bonded to said substrate surface with a refractive index matching transparent glue.
22. The integral portion of a biological reaction vessel according to claim 16, wherein said transfer film carrier includes a negative draft such that a distal diameter defined by said surface of said transfer film carrier is greater than a proximal diameter defined by an inner perimeter of said transfer film carrier.
23. The integral portion of a biological reaction vessel according to claim 22, wherein said transfer film carrier includes a girdle that is contiguous with said negative draft.
24. The integral portion of a biological reaction vessel according to claim 22, wherein said transfer film carrier includes a chamfer that is contiguous with said substrate surface.
25. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film has a thickness that is less than 500 microns.

26. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film has a thickness that is held to within 20%.
27. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film has a surface opposite said substrate surface having a flatness that is held within five microns.
28. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film includes at least one pedestal that protrudes and defines a laser capture microdissection acquisition zone.
29. The integral portion of a biological reaction vessel according to claim 16, wherein said laser capture microdissection transfer film includes a protruding feature that runs along at least at least three points of a perimeter of said laser capture microdissection transfer film.
30. A microcentrifuge tube cap comprising the integral portion of a biological reaction vessel according to claim 16.
31. A laser capture microdissection assembly comprising:
 - a plate having a top surface; and
 - at least one laser capture microdissection cap coupled to said top surface of said plate, wherein said at least one laser capture microdissection cap includes
 - a transfer film carrier having a substrate surface; and
 - a laser capture microdissection transfer film coupled to said substrate surface of said transfer film carrier.
32. The laser capture microdissection assembly of claim 31, further comprising a release layer coated on said plate, said release layer being located between said plate and said laser capture microdissection transfer film of each of said at least one laser capture microdissection cap.

33. The laser capture microdissection assembly of claim 32, wherein said release layer includes at least one nonadhesive material selected from the group consisting of silicones and polytetrafluoroethylenes.
34. The laser capture microdissection assembly of claim 33, wherein said at least one nonadhesive material is a silicone containing surfactant agent.
35. The laser capture microdissection assembly of claim 31, wherein a plano-concave void is located between said laser capture microdissection transfer film of said at least one laser capture microdissection cap and said top surface of said plate.
36. The laser capture microdissection assembly of claim 31, wherein said laser capture microdissection transfer film includes a transparent thermoplastic.
37. The laser capture microdissection assembly of claim 31, wherein said laser capture microdissection transfer film includes an absorptive substance.
38. The laser capture microdissection assembly of claim 31, wherein said laser capture microdissection transfer film is hot vacuum baked onto said substrate surface.
39. The laser capture microdissection assembly of claim 31, wherein said transfer film carrier includes a negative draft such that a distal diameter defined by said surface of said transfer film carrier is greater than a proximal diameter defined by an inner perimeter of said transfer film carrier.
40. The laser capture microdissection assembly of claim 31, wherein said laser capture microdissection transfer film has a thickness that is less than 500 microns.
41. The laser capture microdissection assembly of claim 31, wherein said laser capture microdissection transfer film has a thickness that is held to within 20% of a given value.

42. The laser capture microdissection assembly of claim 31, wherein said laser capture microdissection transfer film has a surface opposite said substrate surface having a flatness that is held within five microns.
43. The laser capture microdissection assembly of claim 31, further comprising at least one diffuser coupled to said at least one transfer film carrier.
44. A set of microcentrifuge tube caps comprising the laser capture microdissection assembly of claim 31.
45. A method of imaging a sample with a microscope, comprising:
 - providing said microscope;
 - locating a scattering media within a beam path defined by said microscope and within a few millimeters of a sample; and
 - imaging said sample through said scattering media with said microscope.
46. The method of imaging a sample with a microscope according to claim 45, wherein said scattering media is optically coupled to a laser capture microdissection film.
47. A microscope, comprising:
 - a scattering media located within a beam path defined by said microscope and within a few millimeters of a sample.
48. The microscope of claim 47, further comprising a laser capture microdissection film optically coupled to said scattering media.

ABSTRACT OF THE DISCLOSURE

Systems and methods for acquiring laser capture microdissection samples are disclosed. An integral portion of a biological reaction vessel includes a transfer film carrier having a substrate surface; and a laser capture microdissection transfer film coupled to the substrate surface of the transfer film carrier. The systems and methods facilitate quick and accurate laser capture microdissection while simultaneously minimizing contamination.

FIG. 1B

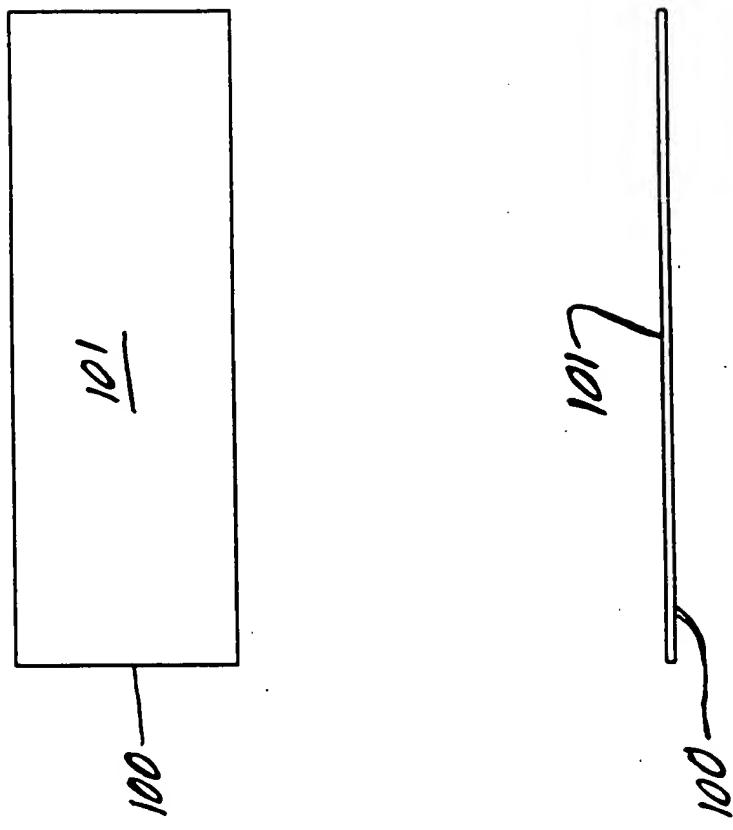


FIG. 1C

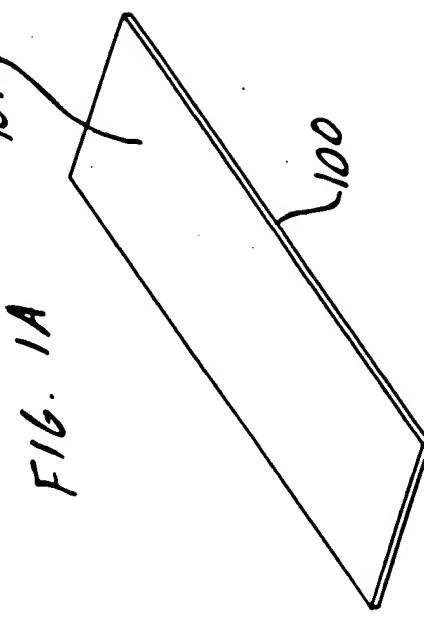


FIG. 1A

F16. 2B



F16. 2A 2107



2107

100 101

F16. 2C

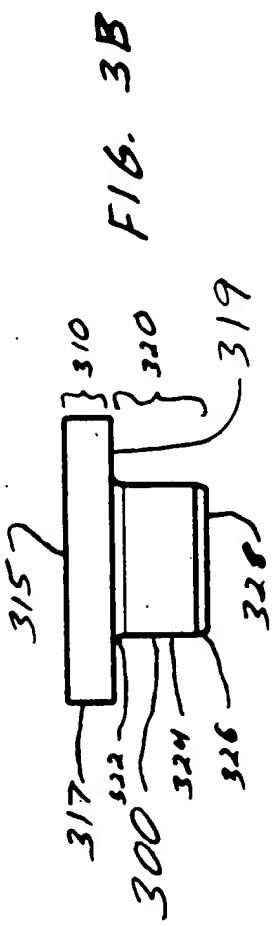
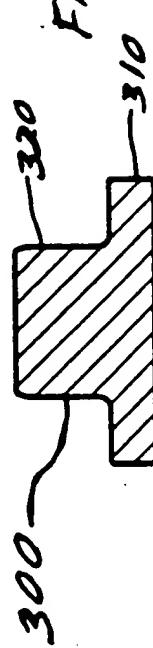
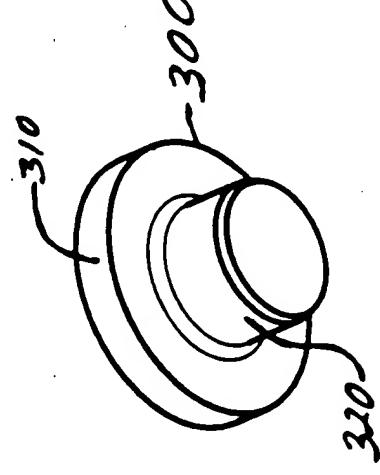
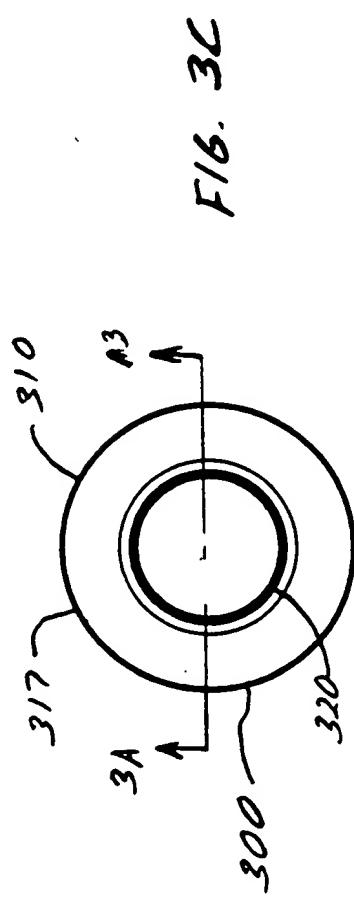
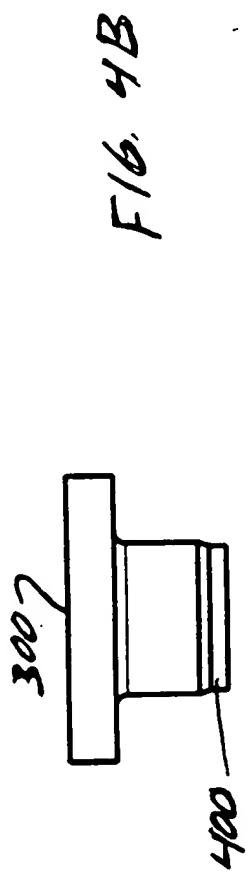
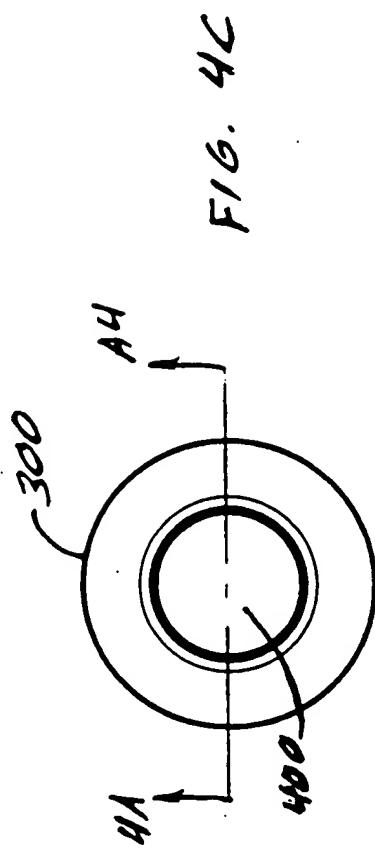
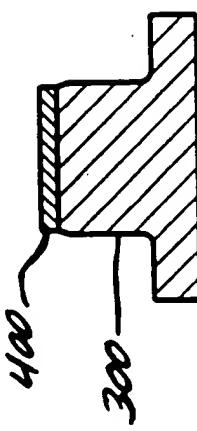
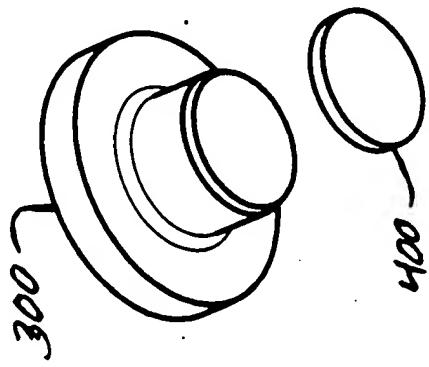


Fig. 3A



SECTION 3A-A3

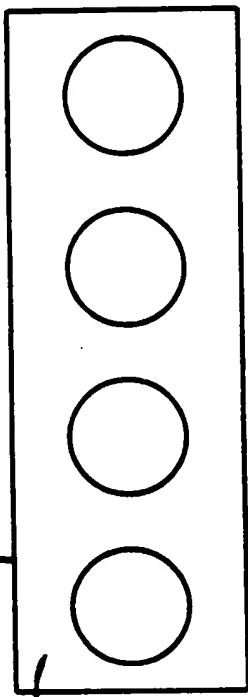
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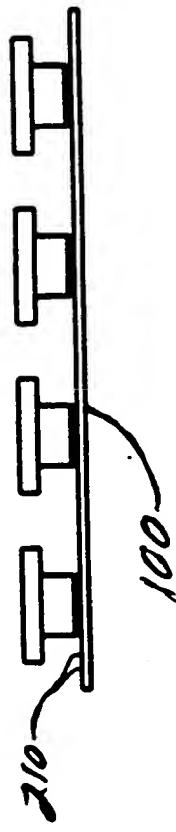
F16 5B

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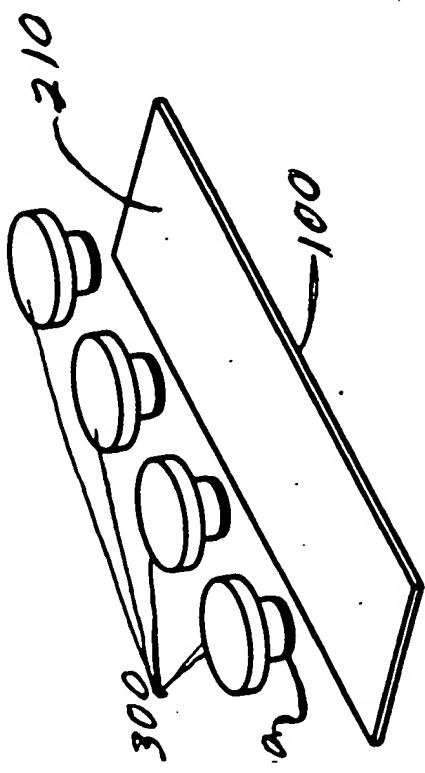
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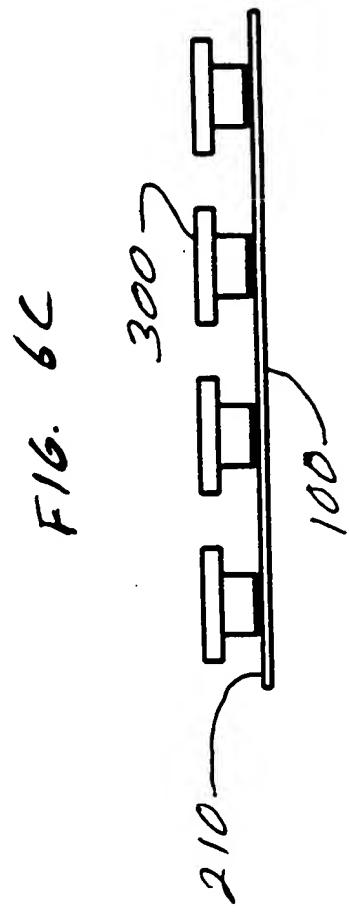
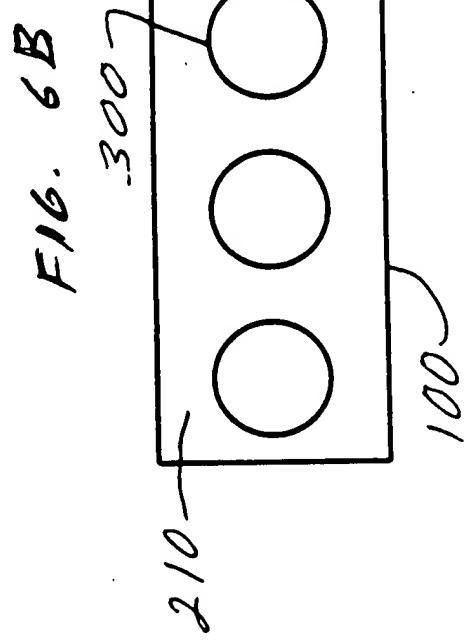
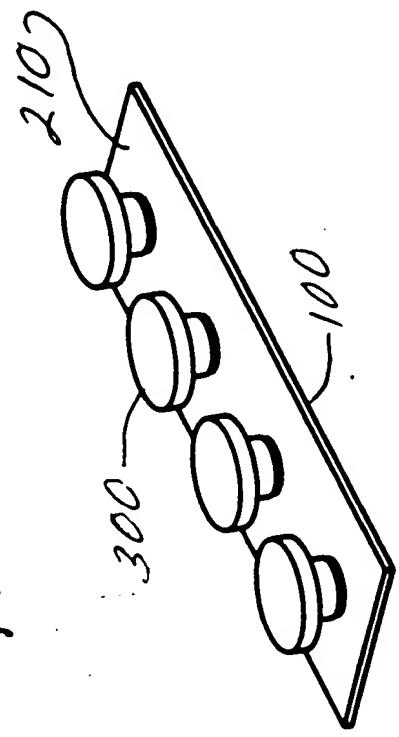


F16 5C



F16. 5A





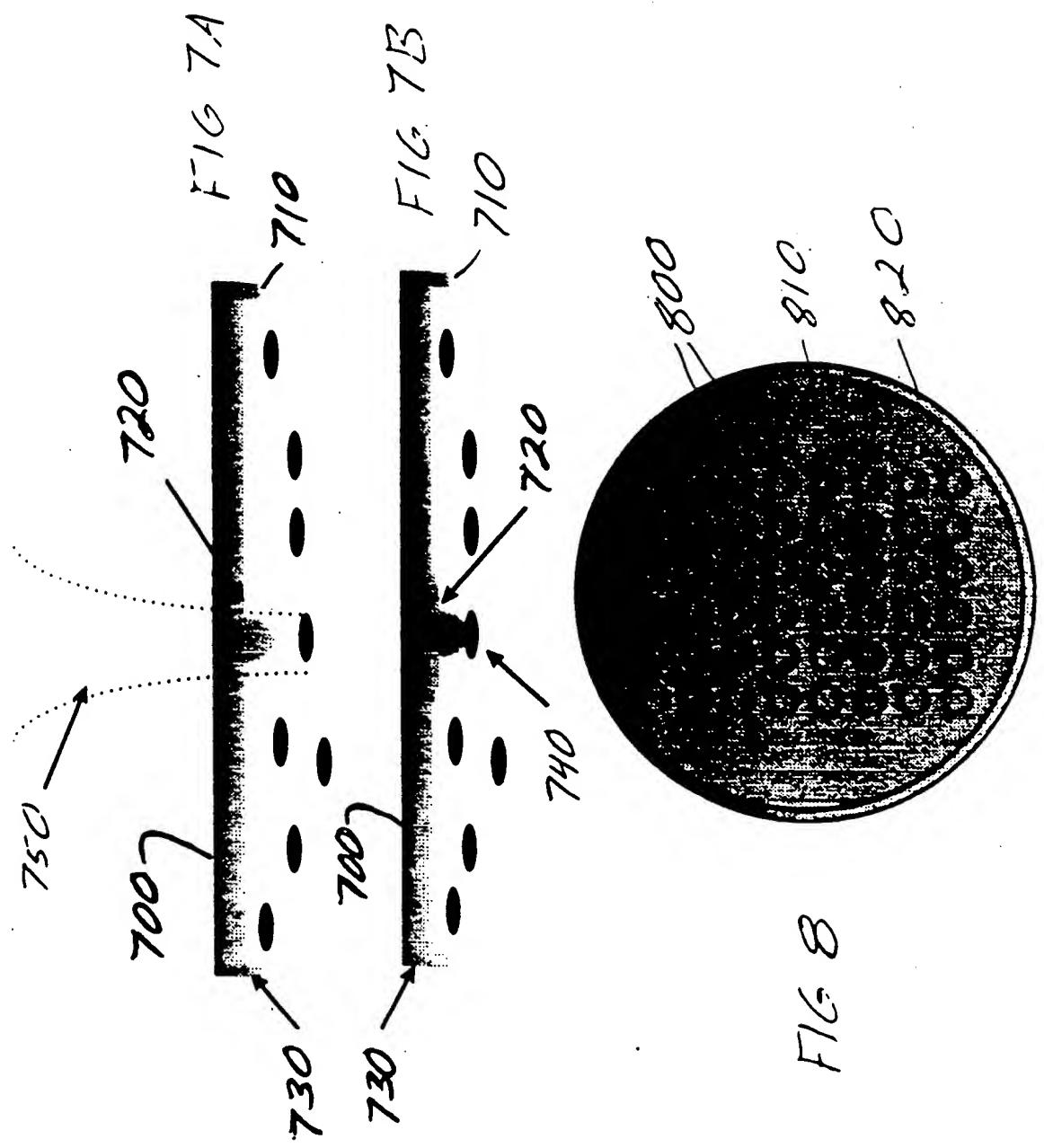


FIG. 9

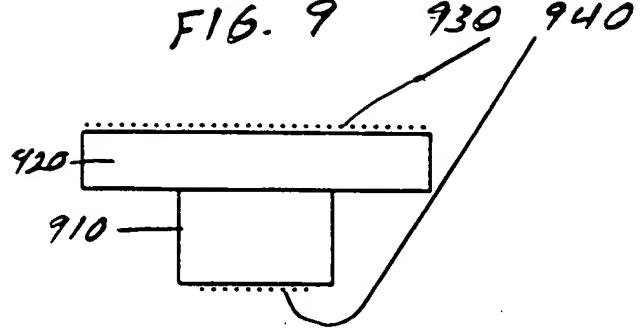
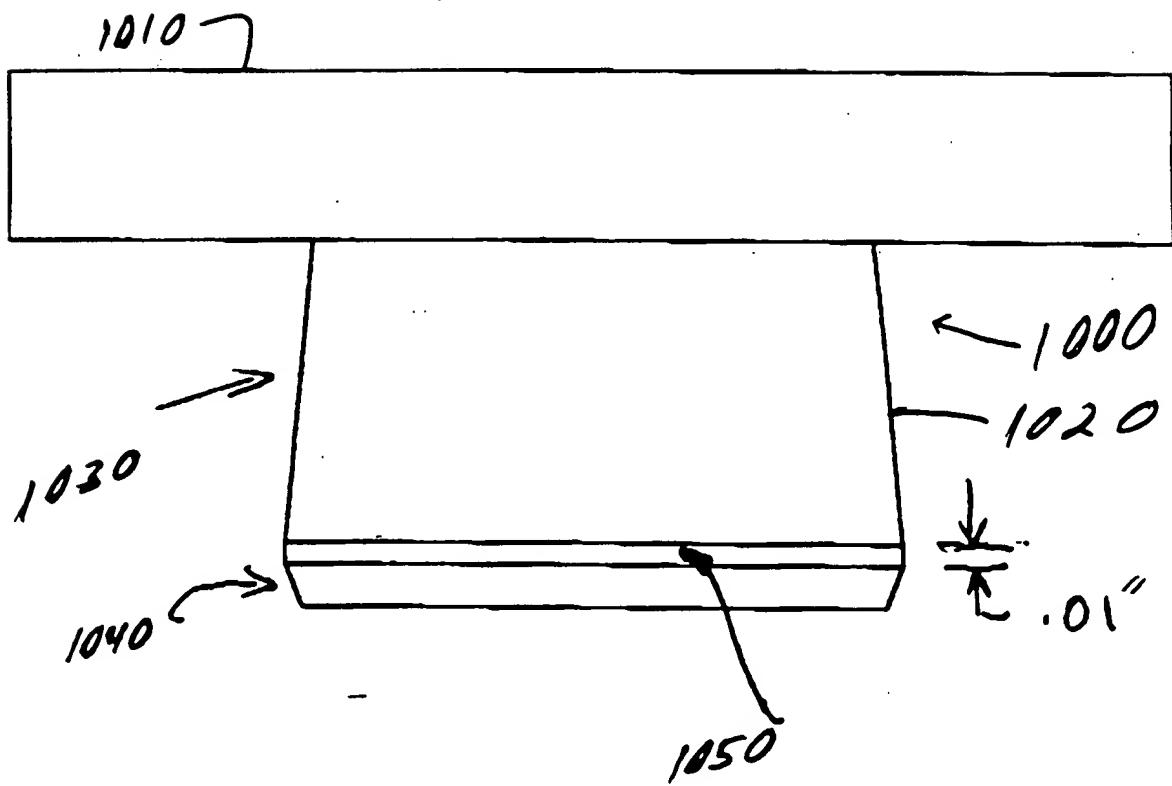
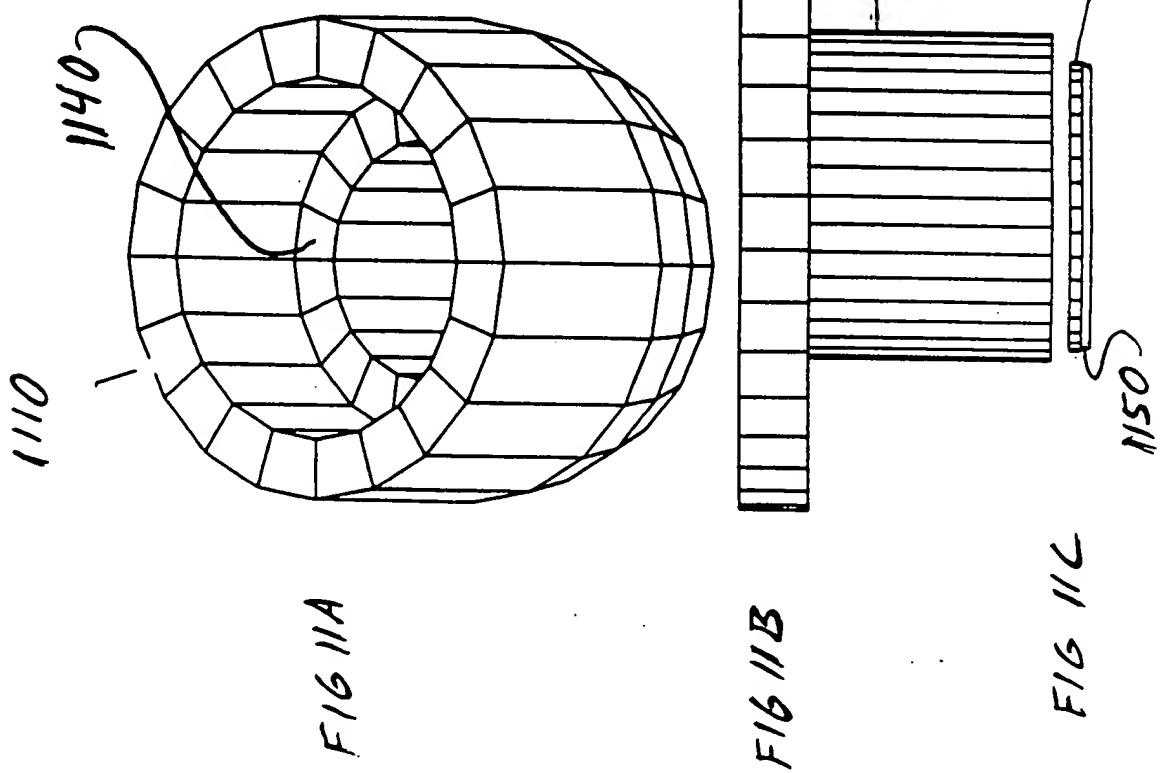
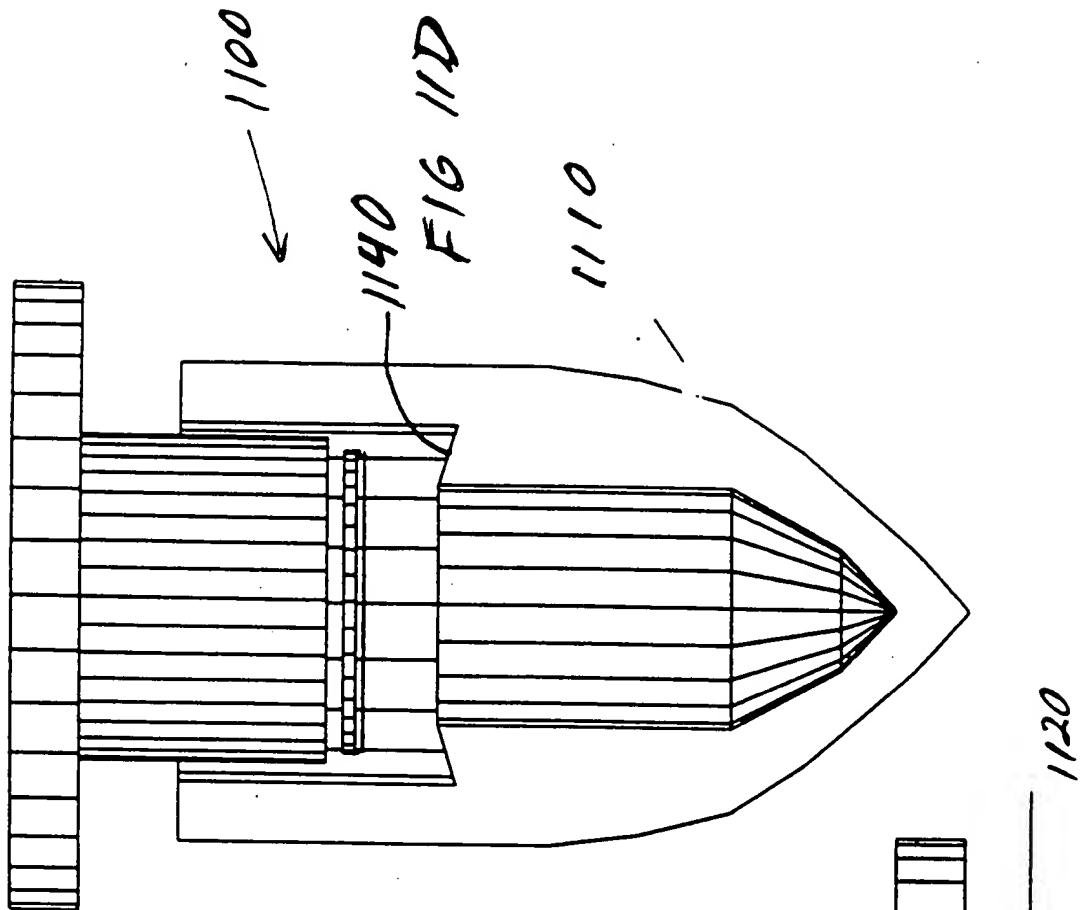


FIG. 10





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09/018,452

UNITED STATES PATENT APPLICATION
ENTITLED

**LASER CAPTURE MICRODISSECTION
METHOD AND APPARATUS**

Inventors: Thomas M. Baer, Mark A. Enright,
David F. Head, Christopher E. Todd

Citizenship: All of the United States

ABSTRACT

Systems and methods for laser capture microdissection are disclosed. A method of laser capture microdissection includes providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument; providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface; placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film; and then transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film. The systems and methods provide the advantages of increased speed and much lower rates of contamination.

PATENT
Attorney Docket No. 17726-706

**LASER CAPTURE MICRODISSECTION
METHOD AND APPARATUS**

Inventors: Thomas M. Baer; Mark A. Enright
David F. Head; and Christopher E. Todd

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part under 35 U.S.C. § 120 of copending U.S. Ser. No. 60/037,864, filed February 7, 1997, and of U.S. Ser. No. 60/060,731, filed October 1, 1997, both now pending, the entire contents of which are hereby incorporated herein by reference as if fully set forth herein.

BACKGROUND OF THE INVENTION

10 **Field of the Invention**

The invention relates generally to the field of laser capture microdissection (LCM). More particularly, the invention relates to inverted microscopes that include specialized apparatus for performing LCM. Specifically, a preferred implementation of the invention relates to an inverted microscope that includes a cap handling subsystem, an illumination/laser optical subsystem, a vacuum chuck subsystem, and a manual joystick subsystem. The invention thus relates to inverted microscopes of the type that can be termed laser capture microdissection inverted microscopes.

Discussion of the Related Art

- Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.
- A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.
- In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes localized heating of the plastic film as it passes through it, imparting to it an

adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue section.

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By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

15

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, *in situ* breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

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Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research. For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that

5 overlaps with laser emitters used to selectively melt the film. The dye is mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

While the films employed in LCM applications have proved

10 satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

The organic dyes which are used to alter the absorption

15 characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

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SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for

25 laser capture microdissection. There is also a particular need for an improved method of laser capture microdissection.

A first aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection method, comprising: providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument;

5 providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface; placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and then transferring a portion of said sample to said laser capture microdissection transfer film,

10 without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.

15 A second aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection instrument, comprising: an inverted microscope including: an illumination/laser optical subsystem; a translation stage coupled to said illuminator/laser optical subsystem; a cap handling subsystem coupled to said translation stage; a vacuum chuck subsystem coupled to said translation stage; and a manual joystick subsystem coupled to said translation stage.

20 These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details

thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

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BRIEF DESCRIPTION OF THE DRAWINGS

A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

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FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention;

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1;

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

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FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention;

FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention;

5 FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6;

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention;

10 FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8;

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention;

15 FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10;

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention;

FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention;

20 FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention;

25 FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention;

FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention;

5 FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention; and

FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

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The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

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The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997, entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

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Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for

the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is picked and placed and while a translation stage 145 is manipulated in an X-

Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement. Further, this simultaneous movement can be effected with in a single handed manner. The acquisition of samples is thus made quicker.

Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a function of operator hand movement.

In addition, the joystick provides tactile feedback not available with electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X and Y) as a function of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185.

5 After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing

10 lens 190 also adjusts the beam spot size.

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Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be appreciated from FIG. 2B that the illumination beam path includes delivery of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

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25 Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a

mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then
5 passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of
10 the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which can include a camera. In idle mode, the laser beam path 310 provides a visible low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers
15 energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second,
20 preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength of the laser is 810 nanometers. In a preferred embodiment the spot size of the laser at the EVA material located on microcentrifuge tube cap 120 is variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more
25 preferably from 5 to 30 microns. These ranges are relatively preferred when designing the optical subsystem. From the standpoint of the clinical

operator, the widest spot size range is the most versatile. A lower end point in the spot size range on the order of 5 microns is useful for transferring single cells.

5 Suitable lasers can be selected from a wide power range. For example, a 100 watt laser can be used. On the other hand, a 50 mW laser can be used. The laser can be connected to the rest of the optical subsystem with a fiber optical coupling. Smaller spot sizes are obtainable using diffraction limited laser diodes and/or single mode fiber optics. Single mode fiber allows a diffraction limited beam.

10 While the laser diode can be run in a standard mode such as TEM_{00} , other intensity profiles can be used for different types of applications. Further, the beam diameter could be changed with a stepped lens instead of lens 350.

15 Changing the beam diameter permits the size of the portion of the sample that is acquired to be adjusted. Given a tightly focused initial condition, the beam size can be increased by defocusing. Given a defocused initial condition, the beam size can be decreased by focusing. The change in focus can be in fixed amounts. The change in focus can be obtained by means of indents on a movable lens mounting and/or by means of optical glass steps. In any event, increasing/decreasing the optical path length is the effect that is needed to alter the focus of the beam, thereby altering the spot size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

beam
diameter

20 25 Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this

embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic), and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial.

Fluorescence emitted by the sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for damping vertical motion of the cap handling mechanic subassembly 110. The dampener 510 is adapted to lower the microcentrifuge tube cap 120

down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable of retarding the vertical motion of the subassembly 110 so as not to generate an impulse. As

5 the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before

10 the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to

15 microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube

20 cap 120 and then the microcentrifuge tube cap 120 is picked up off of the slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

25 The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel

to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 off the slide (not shown).

The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture microdissection transfer film is approximately 0.26 square inches.

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pressure

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

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Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to the position depicted in FIGS. 6-7).

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Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into

a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place.

Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

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Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

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Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430

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collimates the light from the fiber optic 1410. The collimator lens 1430 can be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens).

A collimated beam 1440 from the collimator lens 1430 then passes though a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460

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is coaxial with the white light illumination. Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG

010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470 can have a numerical aperture of approximately 0.8. This can be
5 characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that
10 contains the cells to be sampled.

More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type
15 eliminates the need for refractive index matching of the sample. Such a scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

The scattering media can be a diffuser material. A diffuser material
20 that is suitable for use as the scattering media is milk or opal glass which is a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example,
25 frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser.
In any event, the scattering media should be a material that aggressively

scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light sufficiently.

5 Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed into the microcentrifuge tube cap 120 so as to acquire the desired sample.

10 The purpose of the illuminator design is to provide a very high numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization, this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important because the sample is adjacent the bottom of the sample carrier and the

15 objective cannot move closer to the sample than the top of the sample carrier.

20 The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in

visualization. This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

5 The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

10 In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

15 Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

20 The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

25 Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light

emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or
5 indirectly connected to the transfer film carrier and/or the LCM transfer
film. Alternatively, the scattering media can be formed on a surface of, or
the interior of, the transfer film carrier and/or the LCM transfer film. The
scattering media can be fabricated so as to shape the LCM beam and/or the
illumination beam. The scattering media needs to be within a few
10 millimeters of the sample to be effective. A few millimeters means less
than one centimeter, preferably less than five millimeters.

The process of operating the instrument begins by visualizing the
tissue from which the sample is to be acquired. The tissue is then moved to
bring the portion that is to be acquired directly below the principal axis of
15 the instrument. A laser capture microdissection transfer film is then set over
the desired area. In a preferred embodiment the film is spaced to within a
few microns of the top surface of the sample. Alternatively, the film can be
placed in contact with the top of the sample with a pressure sufficient to
allow transfer without forcing nonspecific transfer. Finally, the laser is
20 pulsed to heat the film and remove the tissue. The film needs to be pulled
off of the sample quickly. Though the velocity should be such that the
sample is thixotropically sheared.

Practical Applications of the Invention

25 A practical application of the invention that has value within the
technological arts is the collection of a large database of gene expression

patterns of both healthy and diseased tissue, at different stages of diseases. This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually innumerable uses for the invention, all of which need not be detailed here.

Advantages of the Invention

A laser capture microdissection instrument and/or method representing an embodiment of the invention can be cost effective and advantageous for at least the following reasons. The invention will replace current methods with better technology that allows for more accurate and reproducible results. The invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent

was specifically and individually indicated to be incorporated in its entirety by reference.

All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is

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intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
 - 2 providing a sample that is to undergo laser capture microdissection;
 - 3 positioning said sample within an optical axis of a laser capture
 - 4 microdissection instrument;
 - 5 providing a transfer film carrier having a substrate surface and a laser
 - 6 capture microdissection transfer film coupled to said substrate surface;
 - 7 placing said laser capture microdissection transfer film in
 - 8 juxtaposition with said sample with a pressure sufficient to allow laser
 - 9 capture microdissection transfer of a portion of said sample to said laser
 - 10 capture microdissection transfer film, without forcing nonspecific transfer of
 - 11 a remainder of said sample to said laser capture microdissection film; and
 - 12 then
 - 13 transferring a portion of said sample to said laser capture
 - 14 microdissection transfer film, without forcing nonspecific transfer of a
 - 15 remainder of said sample to said laser capture microdissection transfer film.
1. The laser capture microdissection method of claim 1, wherein
2 the step of placing said laser capture microdissection transfer film in
3 juxtaposition with said sample with a pressure sufficient to allow laser
4 capture microdissection transfer of a portion of said sample to said laser
5 capture microdissection transfer film includes contacting said sample with
6 said laser capture microdissection transfer film and then pressing said laser
7 capture microdissection transfer film against said sample with a force.

1 3. The laser capture microdissection method of claim 2, wherein
2 pressing said laser capture microdissection transfer film against said sample
3 is effected with a self leveling weight that exerts said force.

1 4. The laser capture microdissection method of claim 3, wherein
2 pressing said laser capture microdissection transfer film against said sample
3 is effected by lowering said self leveling weight with a damped transfer
4 arm so as to substantially not generate an impulse.

1 5. The laser capture microdissection method of claim 1, wherein
2 transferring includes exposing said laser capture microdissection transfer
3 film to an amount of electromagnetic energy sufficient to adhere said
4 portion of said sample to said laser capture microdissection transfer film,
5 without forcing nonspecific transfer of said remainder of said sample to said
6 laser capture microdissection transfer film.

1 6. The laser capture microdissection method of claim 5, wherein
2 said amount of electromagnetic energy is directed toward said laser capture
3 microdissection transfer film along said optical axis, and, further comprising
4 imaging said sample along said optical axis.

1 7. The laser capture microdissection method of claim 6, wherein
2 said amount of electromagnetic energy is insufficient to significantly
3 denature said portion of said sample.

*no longer
biologically
active*

1 8. The laser capture microdissection method of claim 6, wherein
2 said sample includes at least one member selected from the group consisting
3 of chromophores and fluorescent dyes, and, further comprising identifying
4 at least a portion of said sample with light that excites said at least one
5 member, before the step of transferring said portion of said sample to said
6 laser capture microdissection transfer film.

1 9. The laser capture microdissection method of claim 1, further
2 comprising removing said laser capture microdissection film from said
3 sample, after the step of transferring said portion of said sample to said laser
4 capture microdissection transfer film.

1 10. The laser capture microdissection method of claim 9, wherein
2 the step of removing includes moving both said laser capture
3 microdissection transfer film and said portion of said sample away from said
4 remainder of said sample at a velocity sufficient to thixotropically shear said
5 portion of said sample from said remainder of said sample.

1 11. The laser capture microdissection method of claim 10, further
2 comprising moving both said laser capture microdissection transfer film and
3 said portion of said sample out of said optical axis, after the step of
4 removing said laser capture microdissection transfer film from said sample.

1 12. The laser capture microdissection method of claim 11,
2 wherein said step of moving is effected by a pivot mounted transfer arm,
3 and, further comprising attaching said transfer film carrier to a biological

4 reaction vessel so that both said laser capture microdissection transfer film
5 and said portion of said sample are located in an interior of said biological
6 reaction vessel.

1 13. The laser capture microdissection method of claim 1, further
2 comprising repositioning said sample within said optical axis of said laser
3 capture microdissection instrument, after the step of placing said laser
4 capture microdissection transfer film in juxtaposition with said sample and
5 before the step of transferring.

1 14. The laser capture microdissection method of claim 13,
2 wherein said step of repositioning includes moving said sample in a plane
3 that is substantially perpendicular to said optical axis while said sample is
4 visualized by an operator.

1 15. The laser capture microdissection method of claim 14,
2 wherein said step of repositioning includes repositioning a translation stage
3 that is actuated by an operator with a translation stage joystick.

1 16. The laser capture microdissection method of claim 15,
2 wherein said step of repositioning includes repositioning said translation
3 stage with a manual joystick that is leveraged so as to reduce a movement of
4 the translation stage relative to a movement of a hand of the operator.

1 17. The laser capture microdissection method of claim 1, wherein
2 said sample is provided on a slide and said laser capture microdissection

3 instrument includes a translation stage, and, further comprising holding said
4 slide against said translation stage by actuating a vacuum circuit to exert a
5 force between said slide and said translation stage, before the step of
6 transferring said portion of said sample to said laser capture microdissection
7 transfer film.

1 18. The laser capture microdissection method of claim 17, further
2 comprising modulating said force so as to permit both said slide and said
3 sample to be manually repositioned with regard to said optical axis.

1 19. The laser capture microdissection method of claim 18, further
2 comprising manually repositioning said slide.

1 20. A laser capture microdissection instrument, comprising:
2 a microscope including:
3 an illumination/laser optical subsystem;
4 a translation stage coupled to said illuminator/laser optical
5 subsystem;
6 a transfer film carrier handling subsystem coupled to said translation
7 stage;
8 a vacuum chuck subsystem coupled to said translation stage; and
9 a manual joystick subsystem coupled to said translation stage.

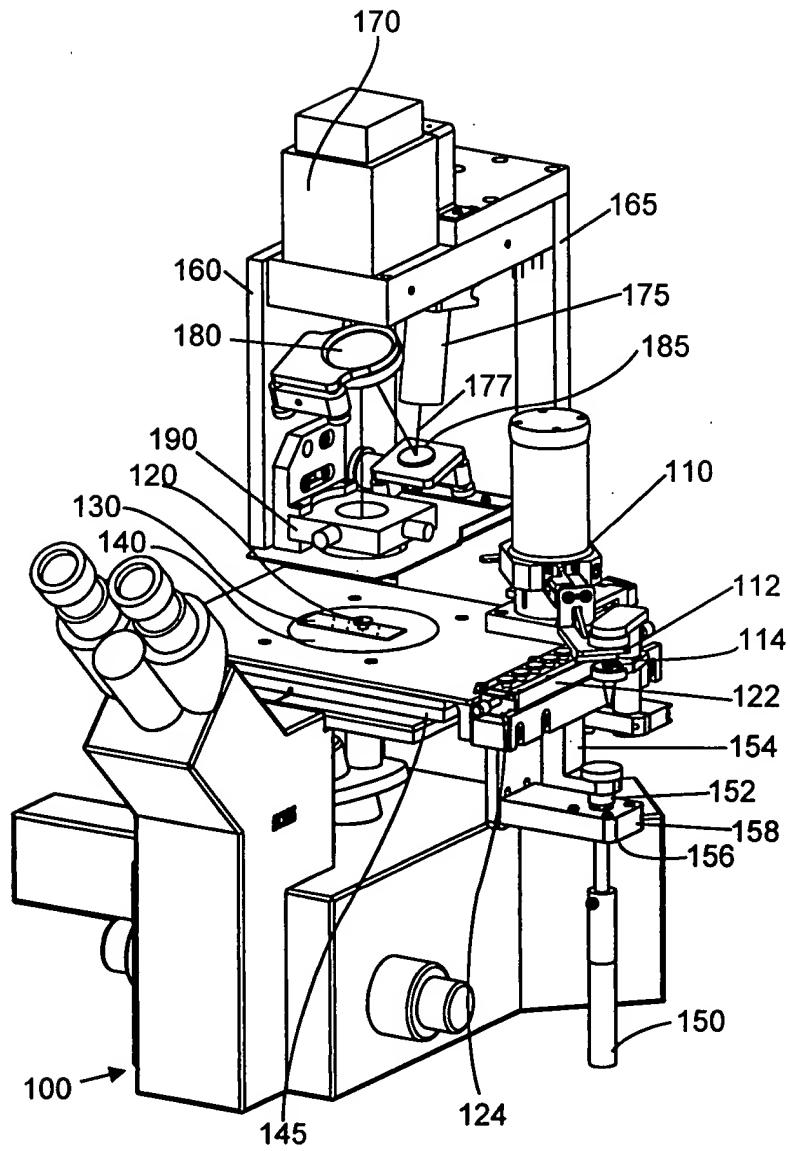
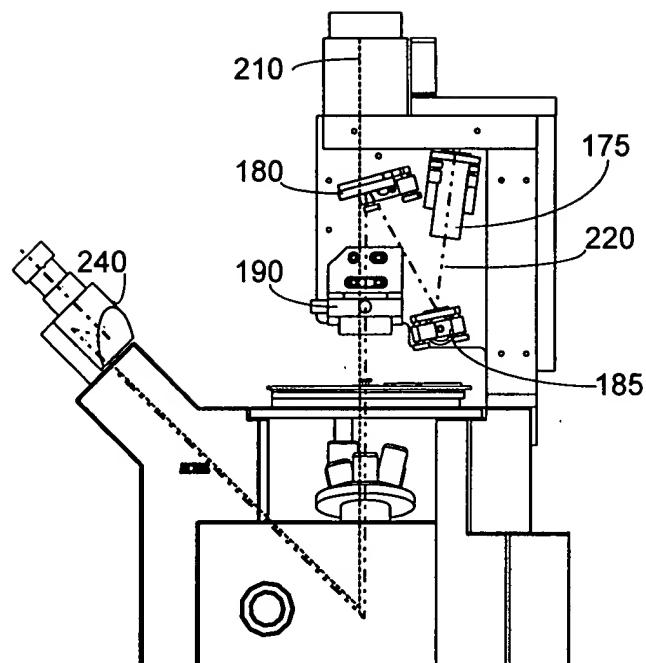
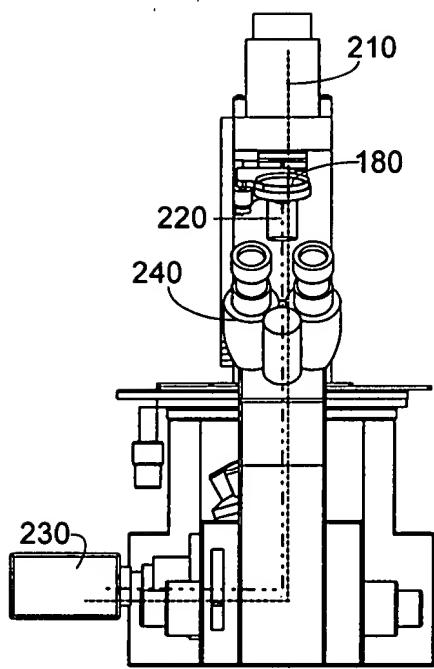


FIG. 1



— White Light Illumination Path
— Laser Beam Path

FIG. 2A

FIG. 2B

FIG. 3

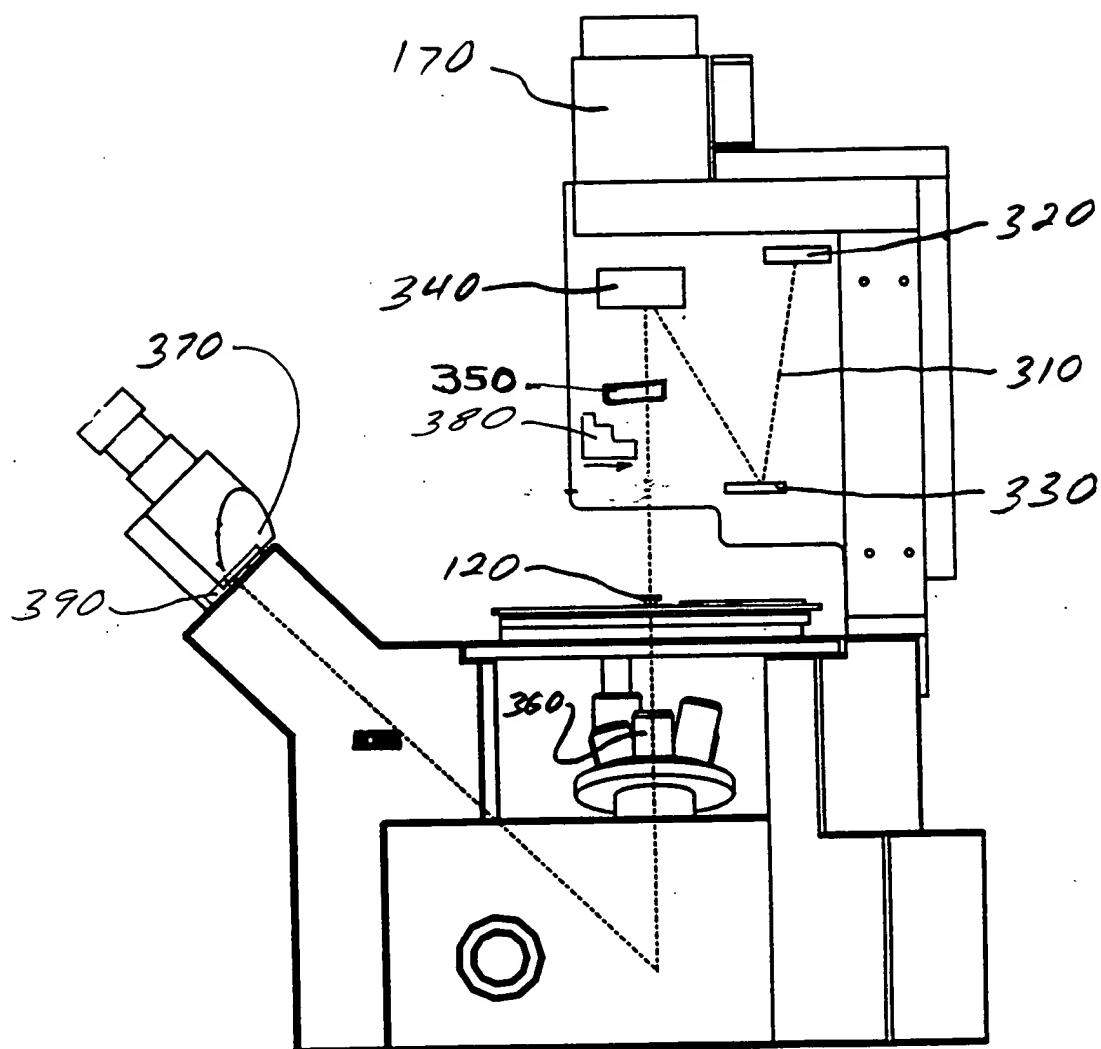
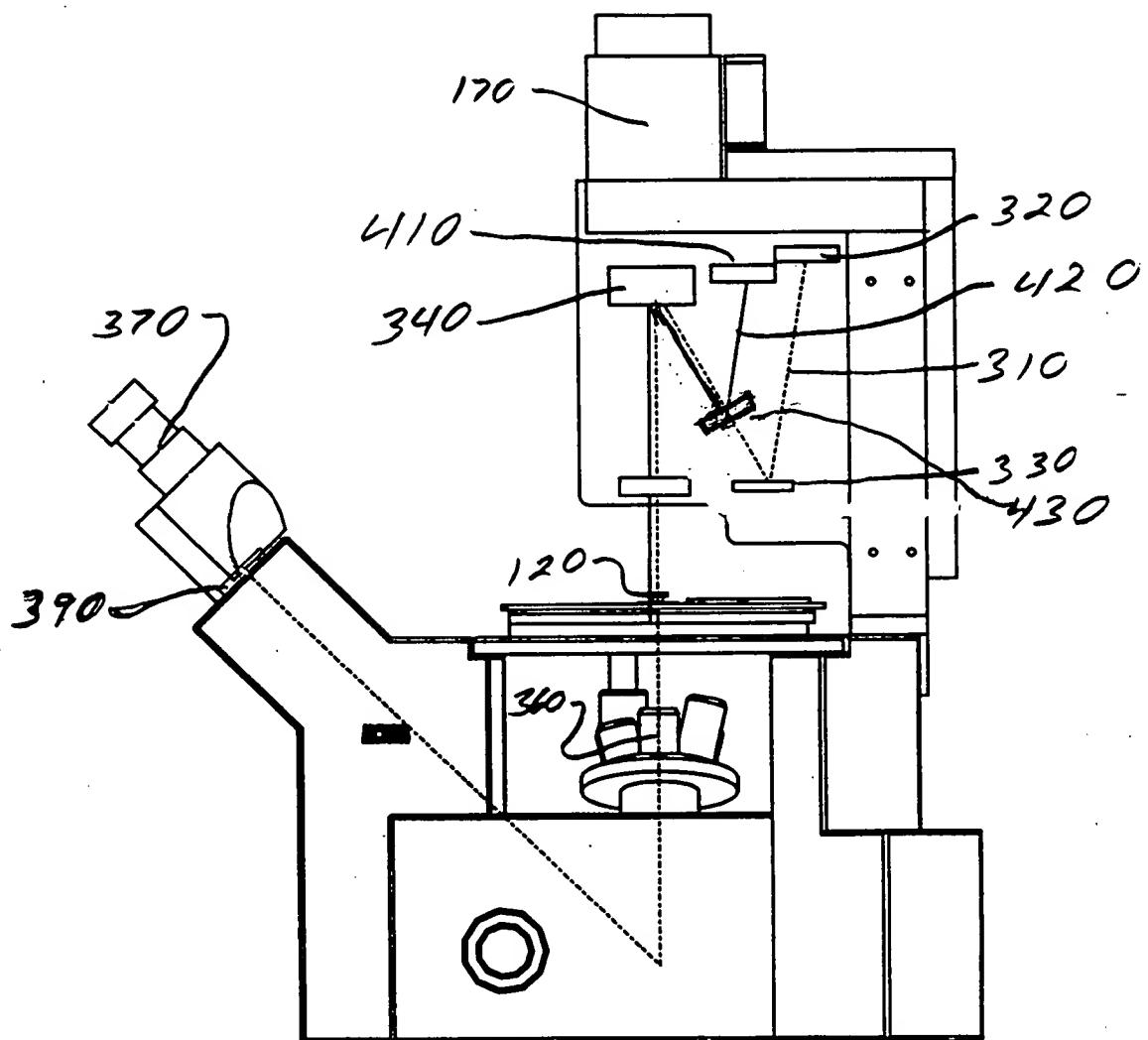


FIG. 4



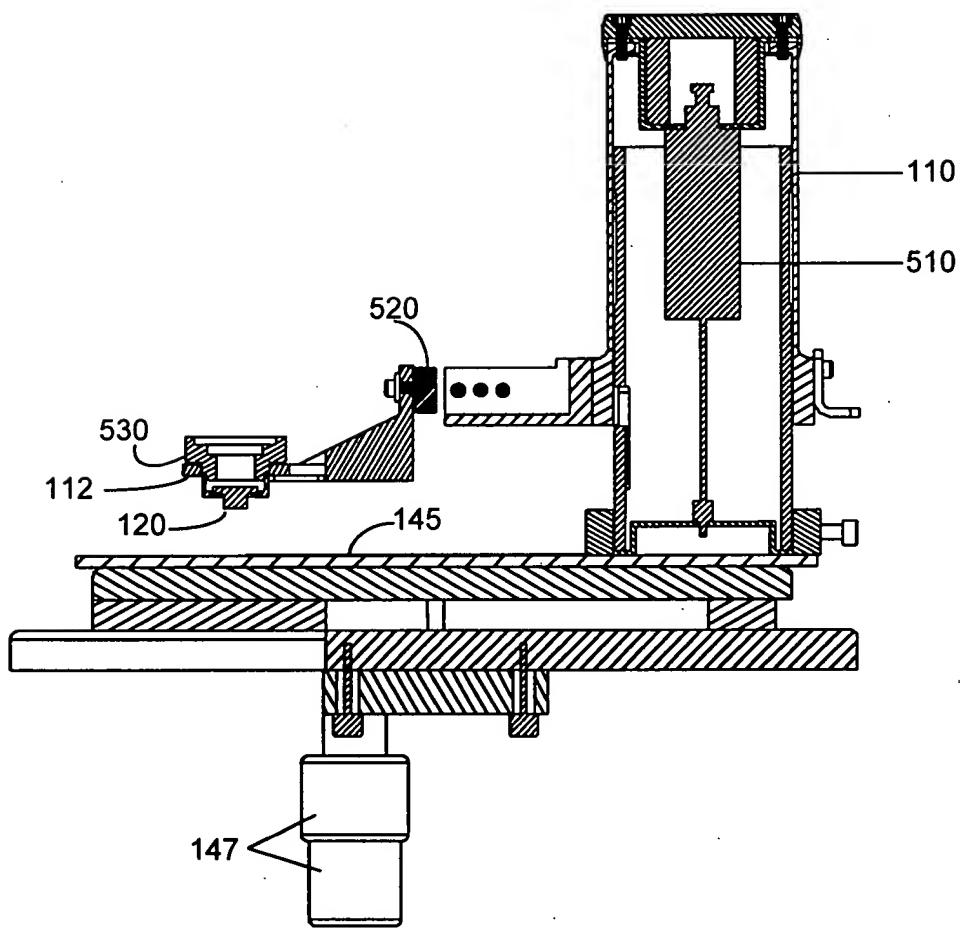


FIG. 5

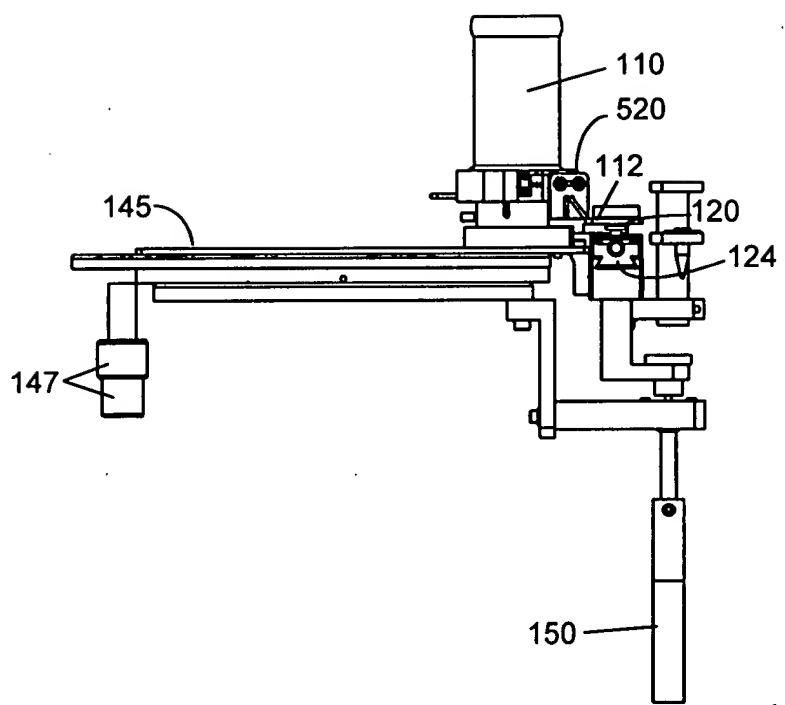


FIG. 6

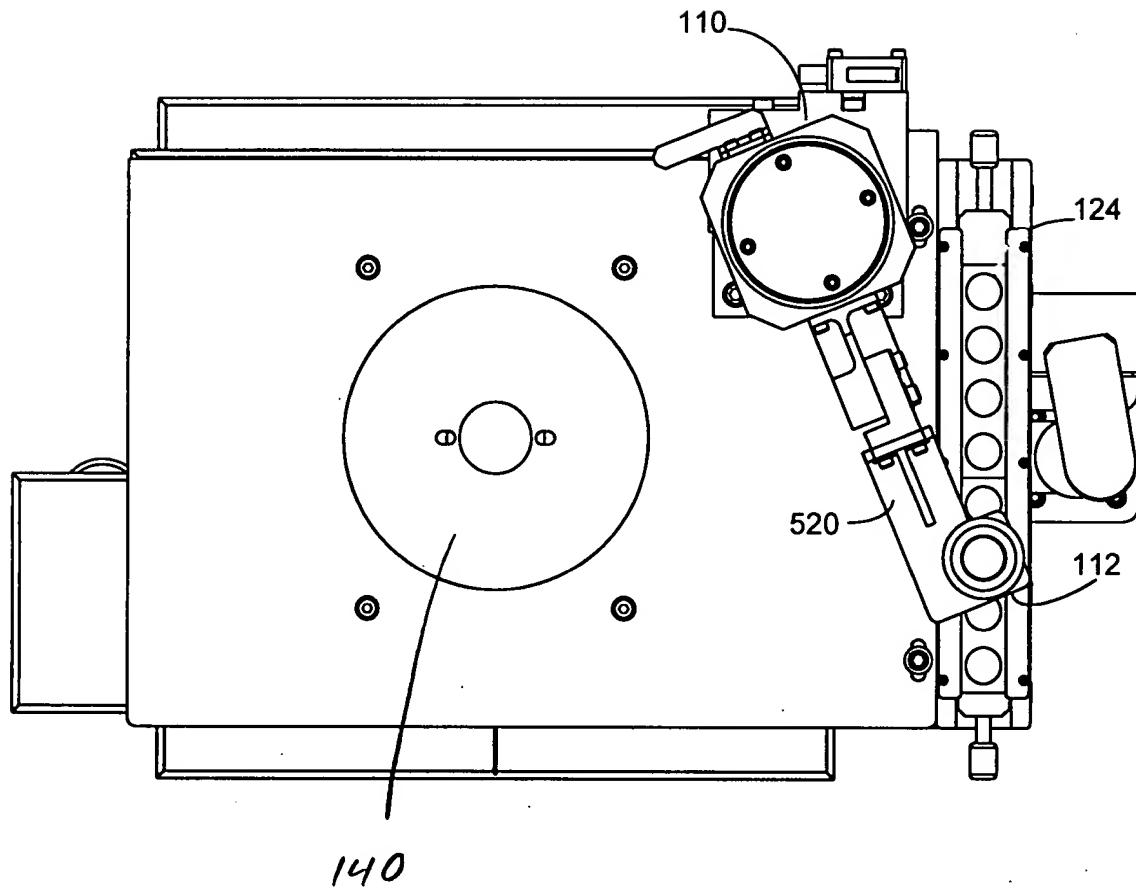


FIG. 7

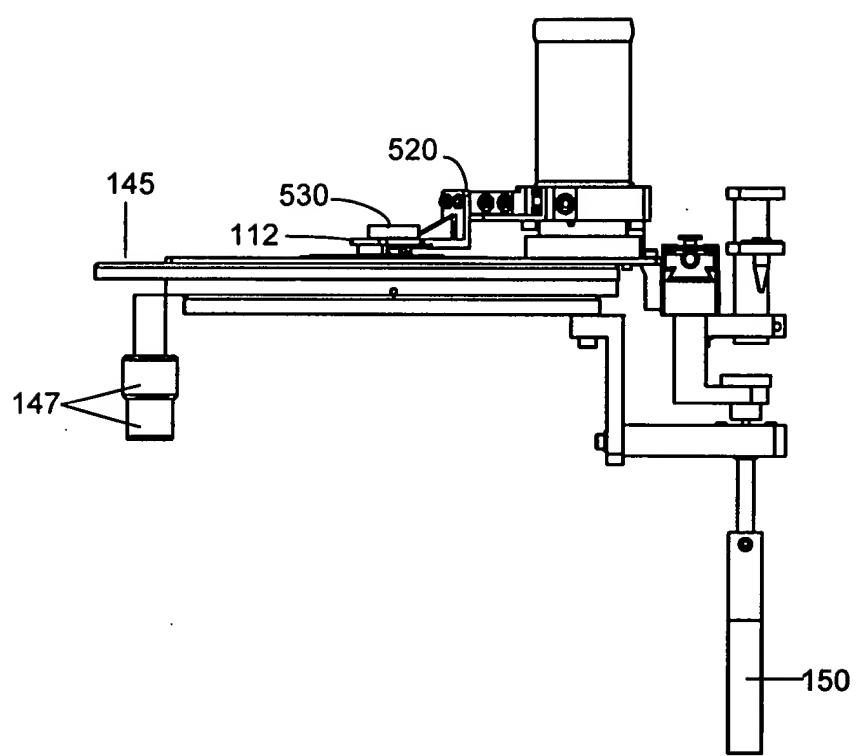


FIG. 8

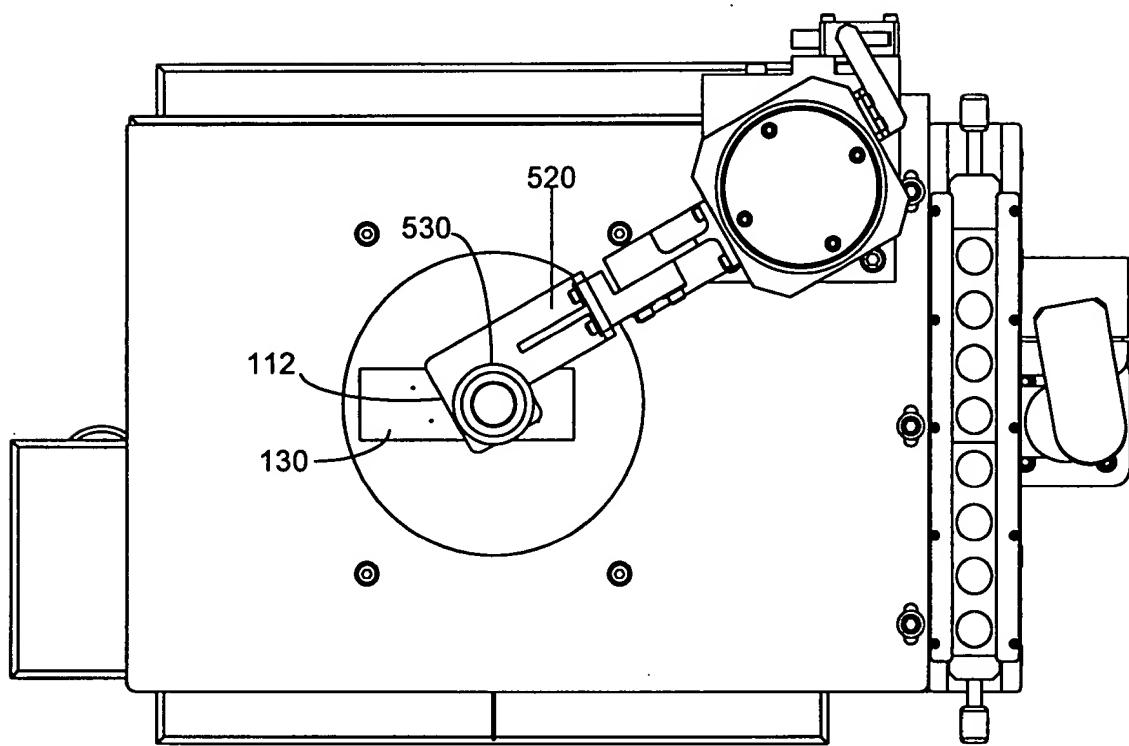


FIG. 9

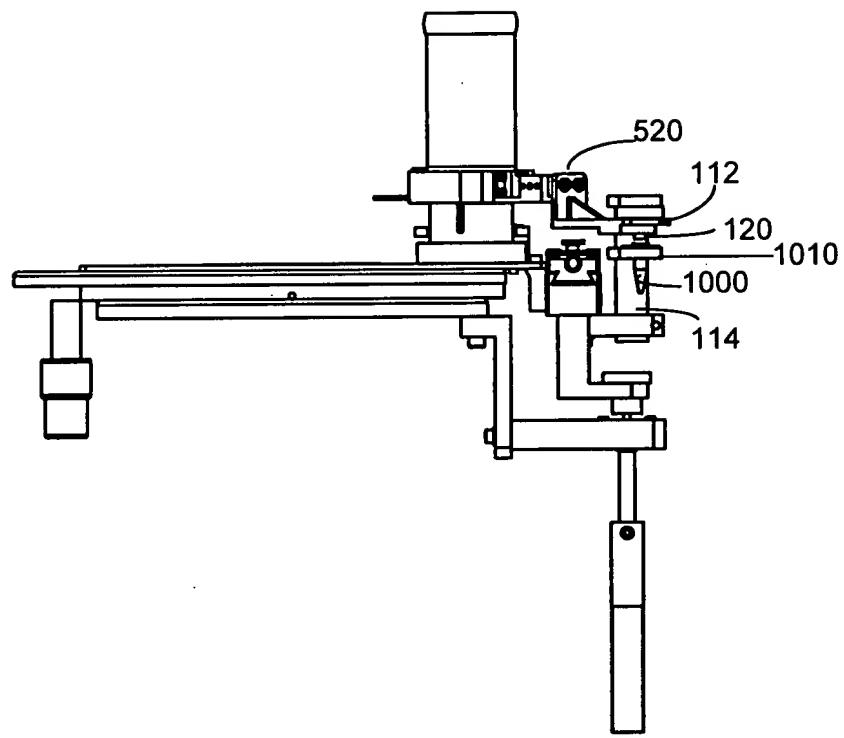


FIG. 10

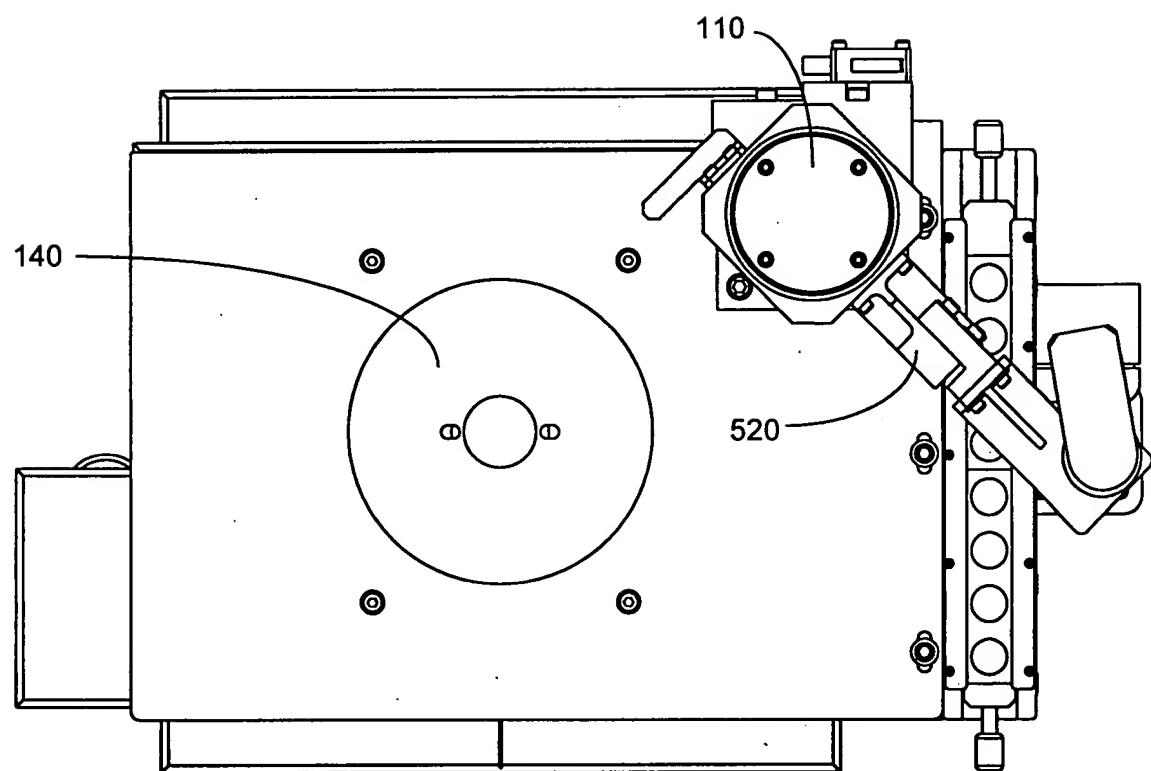


FIG. 11

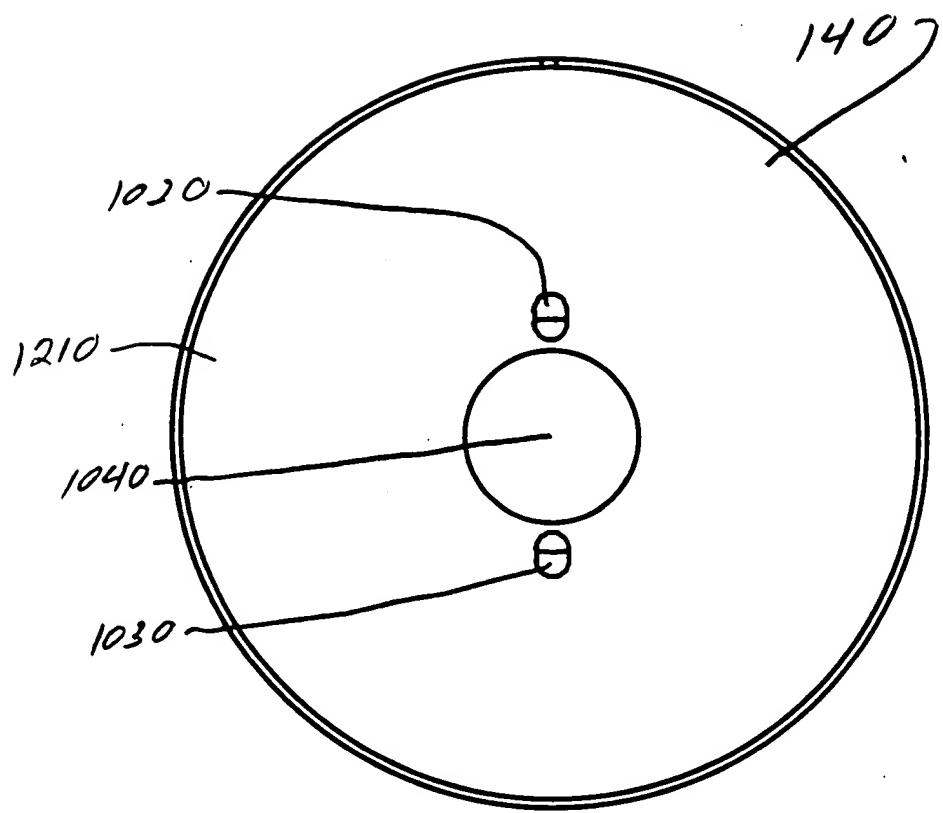


FIG. 12

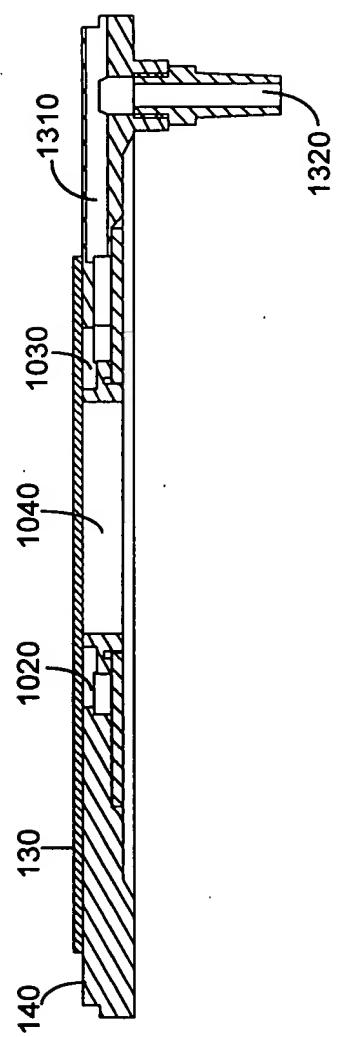


FIG. 13

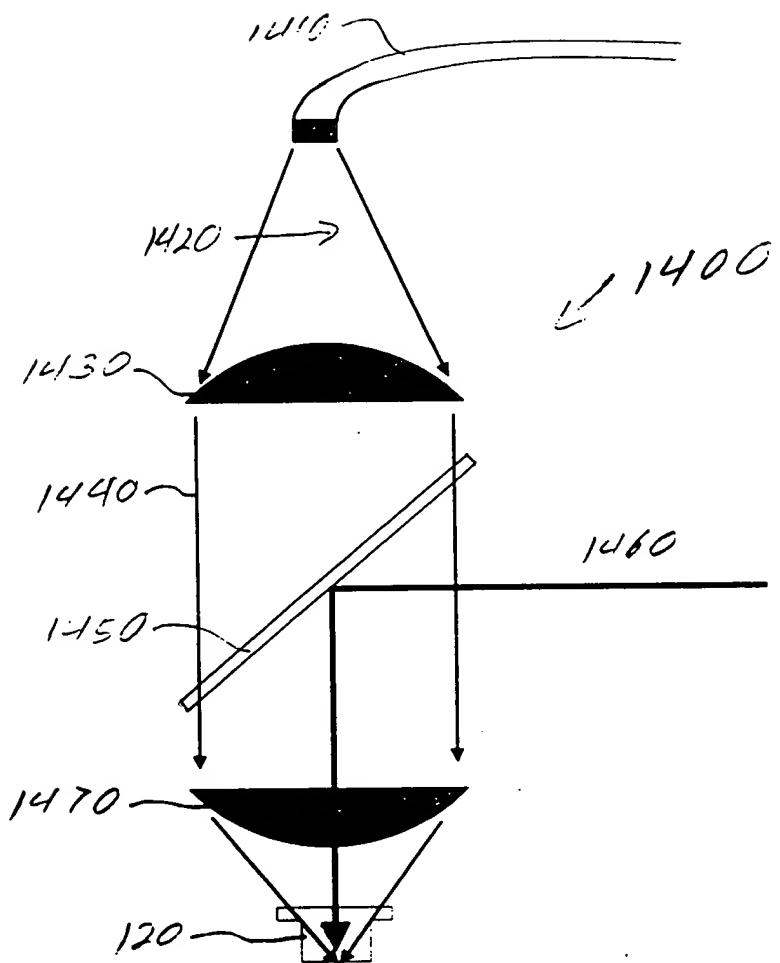


FIG 14

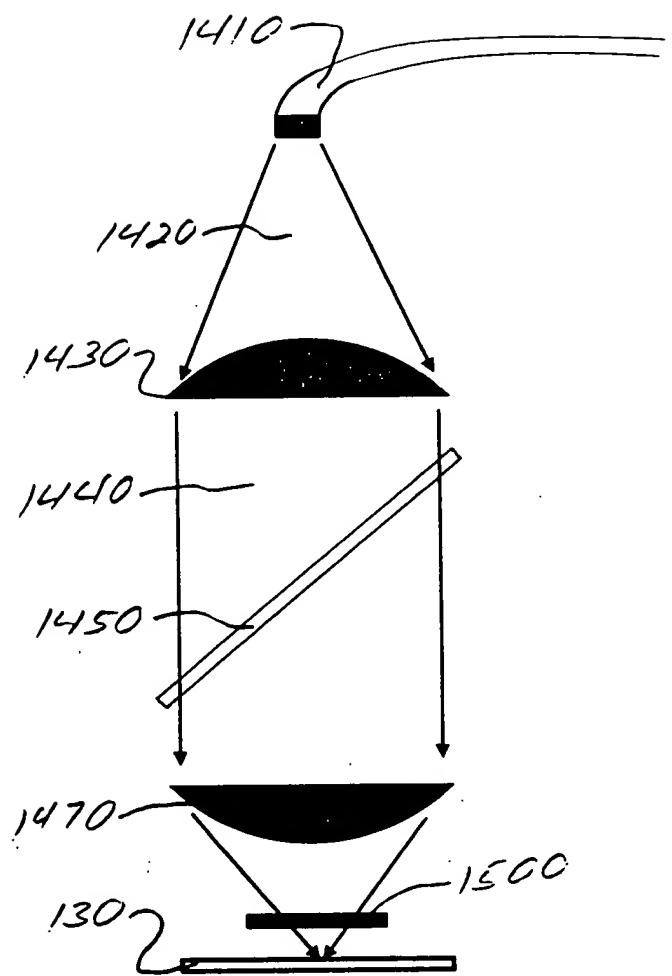


FIG 15

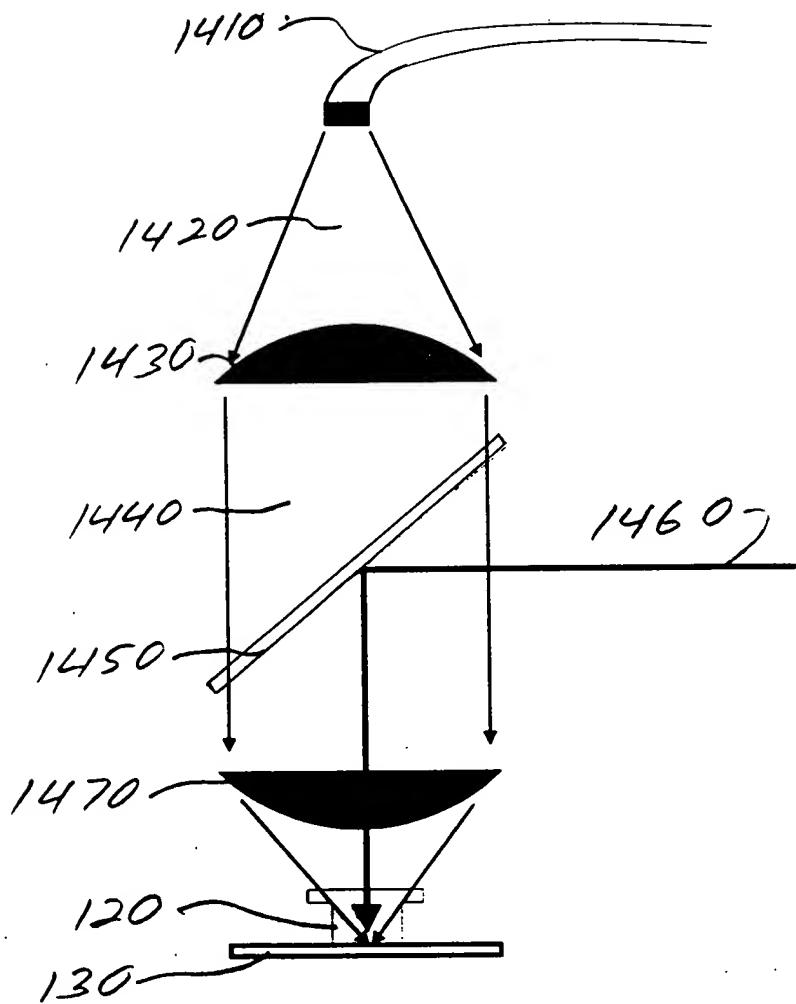


FIG. 16

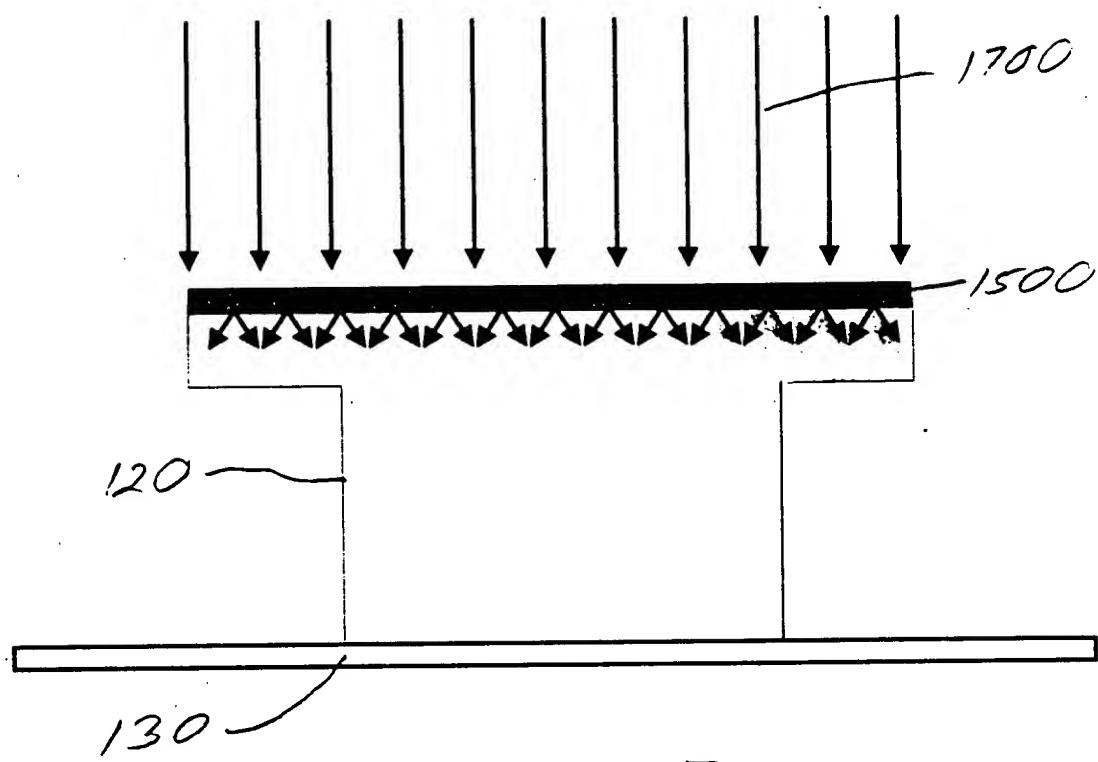


FIG. 17

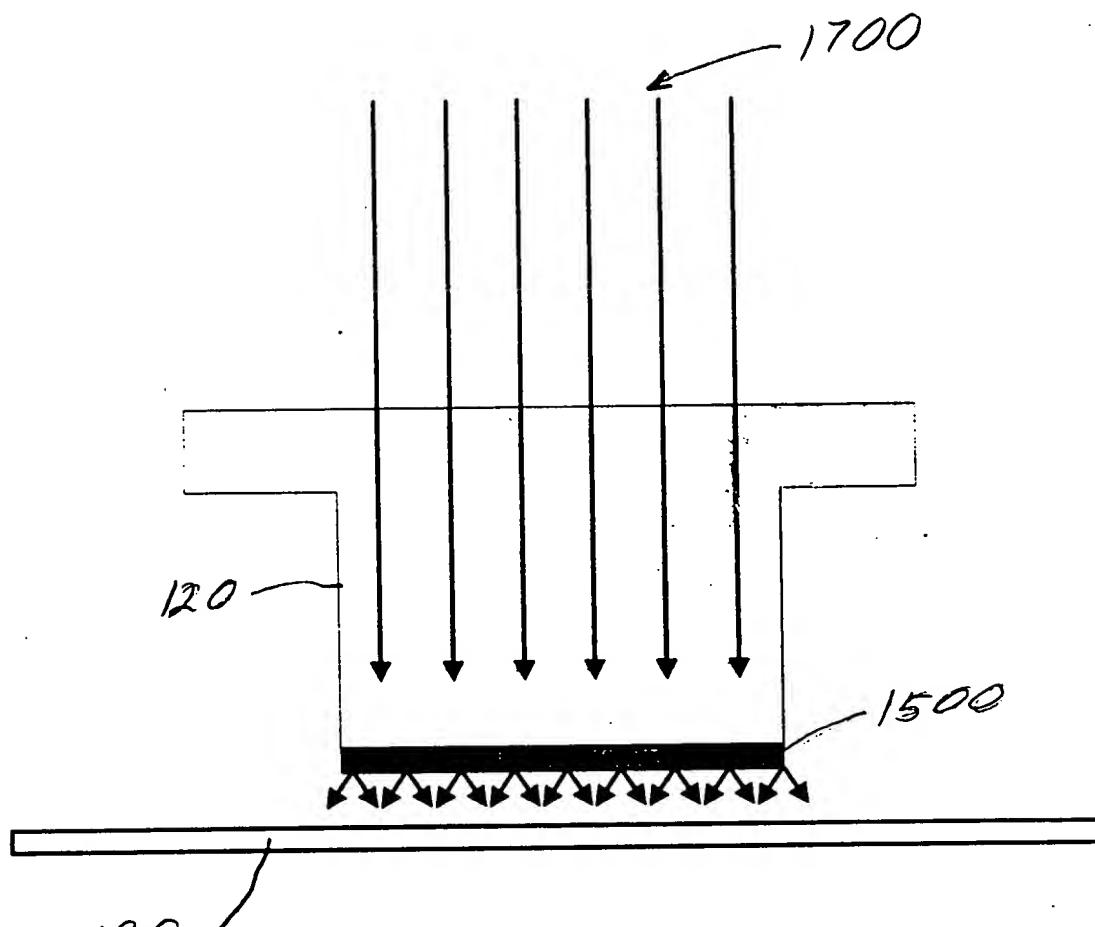


FIG. 18

EM083239913US

UNITED STATES PATENT APPLICATION
ENTITLED

**LASER CAPTURE MICRODISSECTION
PRESSURE PLATE AND TRANSFER ARM**

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David F. Head, Christopher E. Todd

Citizenship: All of the United States

ABSTRACT

Systems and methods for laser capture microdissection are disclosed. A laser capture microdissection method includes: providing a sample that is to undergo laser capture microdissection; positioning the sample within an optical axis of a laser capture microdissection instrument; providing a transfer film carrier removably positioned in a transfer film carrier handling subsystem, the transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to the substrate surface; and then placing the laser capture microdissection transfer film in juxtaposition with the sample by moving the transfer film carrier handling subsystem, the laser capture microdissection transfer film being placed in juxtaposition with a pressure sufficient to allow laser capture microdissection transfer of a portion of the sample to the laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of the sample to the laser capture microdissection film; and then transferring a portion of the sample to the laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of the sample to the laser capture microdissection transfer film. Transferring includes moving the laser capture microdissection transfer film and the portion of the sample away from the remainder of the sample with the transfer film carrier handling subsystem. The transfer film carrier handling subsystem can be mounted on an inverted microscope. The systems and methods provide the advantages of increased speed and much lower rates of contamination.

PATENT
Attorney Docket No. 17726-707

**LASER CAPTURE MICRODISSECTION
PRESSURE PLATE AND TRANSFER ARM**

Inventors: Thomas M. Baer; Mark A. Enright
David F. Head; and Christopher E. Todd

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is, under 35 U.S.C. § 120, a continuation of U.S Ser. No. 09/018,452, filed February 4, 1998, now pending, which is in-turn a continuation-in-part of both U.S. Ser. No. 60/060,731, filed October 1, 5 1997, now pending, and U.S. Ser. No. 60/037,864, filed February 7, 1997, now abandoned, the entire contents of all which are hereby incorporated herein by reference as if fully set forth herein.

BACKGROUND OF THE INVENTION

10 **Field of the Invention**

The invention relates generally to the field of laser capture microdissection (LCM). More particularly, the invention relates to inverted microscopes that include specialized apparatus for performing LCM. Specifically, a preferred implementation of the invention relates to an 15 inverted microscope that includes a cap handling subsystem, an illumination/laser optical subsystem, a vacuum chuck subsystem, and a manual joystick subsystem. The invention thus relates to inverted microscopes of the type that can be termed laser capture microdissection inverted microscopes.

20

Discussion of the Related Art

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

In laser capture microdissection, the operator looks through a microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes localized heating of the plastic film as it passes through it, imparting to it an

5

adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue section.

10

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

15

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ breast carcinoma, atypical ductal hyperplasia of the breast, prostatic interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

20

25

Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research. For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed. The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

5

The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

10

While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

15

The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for laser capture microdissection. There is also a particular need for an improved method of laser capture microdissection.

5 A first aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection method, comprising: providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument; providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface;

10 placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and then transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.

15

20 A second aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection instrument, comprising: an inverted microscope including: an illumination/laser optical subsystem; a translation stage coupled to said illuminator/laser optical subsystem; a cap handling subsystem coupled to said translation stage; a vacuum chuck subsystem coupled to said translation stage; and a manual joystick subsystem coupled to said translation stage.

25

These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

10

BRIEF DESCRIPTION OF THE DRAWINGS

A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

15

FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention;

20

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1;

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

5 FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention;

FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention;

10 FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6;

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention;

FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8;

15 FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention;

FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10;

20 FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention;

FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention;

25 FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention;

FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention;

5 FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention;

FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention; and

10 FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting 15 embodiments that are illustrated in the accompanying drawings and detailed in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

20 The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997 entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if 25 fully set forth herein.

5

Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

10

15

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

20

25

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to

hold the glass slide 130 in place while the microcentrifuge tube cap 120 is picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

5 The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement.
10 Further, this simultaneous movement can be effected with in a single handed manner. The acquisition of samples is thus made quicker.

15 Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a
20 function of operator hand movement.

 In addition, the joystick provides tactile feedback not available with electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X and Y) as a function of a single vector movement of an operator's hand.
25 This important feature provides an unexpected result in that the speed with

which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185.

After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.

Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be appreciated from FIG. 2B that the illumination beam path includes delivery of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which can include a camera. In idle mode, the laser beam path 310 provides a visible low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second, preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength of the laser is 810 nanometers. In a preferred embodiment the spot size of the laser at the EVA material located on microcentrifuge tube cap 120 is

variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more preferably from 5 to 30 microns. These ranges are relatively preferred when designing the optical subsystem. From the standpoint of the clinical operator, the widest spot size range is the most versatile. A lower end point in the spot size range on the order of 5 microns is useful for transferring 5 single cells.

Suitable lasers can be selected from a wide power range. For example, a 100 watt laser can be used. On the other hand, a 50 mW laser can be used. The laser can be connected to the rest of the optical subsystem 10 with a fiber optical coupling. Smaller spot sizes are obtainable using diffraction limited laser diodes and/or single mode fiber optics. Single mode fiber allows a diffraction limited beam.

While the laser diode can be run in a standard mode such as TEM₀₀, other intensity profiles can be used for different types of applications. 15 Further, the beam diameter could be changed with a stepped lens instead of lens 350.

Changing the beam diameter permits the size of the portion of the sample that is acquired to be adjusted. Given a tightly focused initial condition, the beam size can be increased by defocusing. Given a defocused 20 initial condition, the beam size can be decreased by focusing. The change in focus can be in fixed amounts. The change in focus can be obtained by means of indents on a movable lens mounting and/or by means of optical glass steps. In any event, increasing/decreasing the optical path length is the effect that is needed to alter the focus of the beam, thereby altering the spot size. For example, inserting a stepped glass prism 380 into the beam so the 25

beam strikes one step tread will change the optical path length and alter the spot size.

Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic), and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial. Fluorescence emitted by the sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for damping vertical motion of the cap handling mechanic subassembly 110.

5 The dampener 510 is adapted to lower the microcentrifuge tube cap 120 down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable of retarding the vertical motion of the subassembly 110 so as not to generate an impulse. As

10 the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before

15 the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to

20 microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube

25 cap 120 and then the microcentrifuge tube cap 120 is picked up off of the

slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is

lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the microcentrifuge tube cap 120 off the slide (not shown).

5 The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture

10 microdissection transfer film is approximately 0.26 square inches.

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

15 Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the

20 microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to

25 the position depicted in FIGS. 6-7).

Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage

and the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

5 The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

10 There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place.

15 Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

20 Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

25 It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

30 Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430 collimates the light from the fiber optic 1410. The collimator lens 1430 can

be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens). A collimated beam 1440 from the collimator lens 1430 then passes through a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460 is coaxial with the white light illumination. Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG 010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470

5 can have a numerical aperture of approximately 0.8. This can be characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

10

Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that contains the cells to be sampled.

15

More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type

20 eliminates the need for refractive index matching of the sample. Such a scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

25 The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk or opal glass which is

a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example, 5 frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser. In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light 10 sufficiently.

Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed 15 into the microcentrifuge tube cap 120 so as to acquire the desired sample.

The purpose of the illuminator design is to provide a very high numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization, 20 this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important 25 because the sample is adjacent the bottom of the sample carrier and the

objective cannot move closer to the sample than the top of the sample carrier.

The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in visualization. This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a

speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is

pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the velocity should be such that the sample is thixotropically sheared.

5 Practical Applications of the Invention

A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases. This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually innumerable uses for the invention, all of which need not be detailed here.

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Advantages of the Invention

A laser capture microdissection instrument and/or method representing an embodiment of the invention can be cost effective and advantageous for at least the following reasons. The invention will replace current methods with better technology that allows for more accurate and reproducible results. The invention can be used to provide a low cost

injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

5 All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent was specifically and individually indicated to be incorporated in its entirety by reference.

10 All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

15 For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each

disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

It will be manifest that various additions, modifications and
5 rearrangements of the features of the invention may be made without
deviating from the spirit and scope of the underlying inventive concept. It is
intended that the scope of the invention as defined by the appended claims
and their equivalents cover all such additions, modifications, and
rearrangements. The appended claims are not to be interpreted as including
10 means-plus-function limitations, unless such a limitation is explicitly recited
in a given claim using the phrase "means-for." Expedient embodiments of
the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
providing a sample that is to undergo laser capture microdissection;
positioning said sample within an optical axis of a laser capture
microdissection instrument;
providing a transfer film carrier removably positioned in a transfer
film carrier handling subsystem, said transfer film carrier having a substrate
surface and a laser capture microdissection transfer film coupled to said
substrate surface; and then
placing said laser capture microdissection transfer film in
juxtaposition with said sample by moving said transfer film carrier handling
subsystem, said laser capture microdissection transfer film being placed in
juxtaposition with a pressure sufficient to allow laser capture
microdissection transfer of a portion of said sample to said laser capture
microdissection transfer film, without forcing nonspecific transfer of a
remainder of said sample to said laser capture microdissection film; and then
transferring a portion of said sample to said laser capture
microdissection transfer film, without forcing nonspecific transfer of a
remainder of said sample to said laser capture microdissection transfer film,
wherein transferring includes moving said laser capture
microdissection transfer film and said portion of said sample away from said
remainder of said sample with said transfer film carrier handling subsystem.

2. The laser capture microdissection method of claim 1, wherein placing includes

swinging said transfer film carrier handling subsystem toward said optical axis; and then

lowering both said transfer film carrier and said transfer film carrier handling subsystem toward said sample along a direction substantially parallel to said axis.

3. The laser capture microdissection method of claim 2, wherein lowering includes

contacting said sample with said laser capture microdissection transfer film; and then

contacting said transfer film carrier with a weight as to exert said pressure.

4. The laser capture microdissection method of claim 3, wherein

contacting said sample with said laser capture microdissection transfer film includes

retarding vertical motion of said transfer film carrier handling subsystem and

lowering said transfer film carrier at a reproducible rate so that said transfer film carrier undergoes a self-leveling step; and

contacting said transfer film carrier with said weight includes retarding vertical motion of the transfer film carrier handling subsystem; and

lowering said weight at approximately said reproducible rate so that said weight undergoes a self-leveling step.

5. The laser capture microdissection method of claim 1, wherein transferring includes

raising both said transfer film carrier and said transfer film carrier handling subsystem away from said remainder of said sample along a direction substantially parallel to an axis; and then

swinging said transfer film carrier handling subsystem away from said optical axis.

6. The laser capture microdissection method of claim 5, wherein rasing includes

lifting a weight away from said transfer film carrier so as to reduce said pressure; and then

lifting said transfer film carrier and said laser capture microdissection transfer film away from said remainder of said sample.

7. The laser capture microdissection method of claim 6, wherein lifting said weight away from said transfer film carrier includes:

retarding vertical motion of said transfer film carrier handling subsystem; and

raising said weight away from said transfer film carrier at a reproducible rate, and

lifting said transfer film carrier and said laser capture microdissection transfer film away from said remainder of said sample includes:

retarding vertical motion of said transfer film carrier handling subsystem: and

raising said transfer film carrier and said laser capture microdissection transfer film away from said remainder of said sample so that said portion of said sample is thixotropically sheared away from said remainder of said sample.

8. The laser capture microdissection method of claim 1, further comprising, before providing a transfer film carrier removably positioned in a transfer film carrier handling subsystem,

swinging said transfer film carrier handling subsystem to a load position.

9. The laser capture microdissection method of claim 1, further comprising, after transferring said portion of said sample to said laser capture microdissection transfer film,

swinging said transfer film carrier handling subsystem away from said optical axis and toward an unload position; and then

removing said transfer film carrier from said transfer film carrier handling subsystem and joining said transfer film carrier to an analysis container.

10. A laser capture microdissection instrument, comprising:
an illumination/laser optical subsystem defining an optical axis; and
a transfer film carrier handling subsystem mechanically coupled to
said illumination/laser optical subsystem, said transfer film carrier being
adapted to swing a transfer film carrier into and out of said optical axis and
to move said transfer film carrier in a direction substantially parallel to said
optical axis.
11. The laser capture microdissection instrument of claim 10, wherein
said transfer film carrier handling subsystem includes a dampener adapted
to retard movement of the transfer film carrier handling subsystem along
said direction.
12. The laser capture microdissection instrument of claim 10, further
comprising a supply of transfer film carriers.
13. The laser capture microdissection instrument of claim 12, wherein
said supply of transfer film carriers includes a dovetail slide.
14. The laser capture microdissection instrument of claim 10, further
comprising a capping station mechanically coupled to said transfer film
carrier handling subassembly.
15. The laser capture microdissection instrument of claim 10, further
comprising a translation stage mechanically connected to said transfer film
carrier handling subsystem.

16. The laser capture microdissection instrument of claim 15, further comprising a vacuum chuck subsystem mechanically connected to said translation stage.
17. The laser capture microdissection instrument of claim 15, further comprising a manual joystick subsystem mechanically connected to said translation stage.

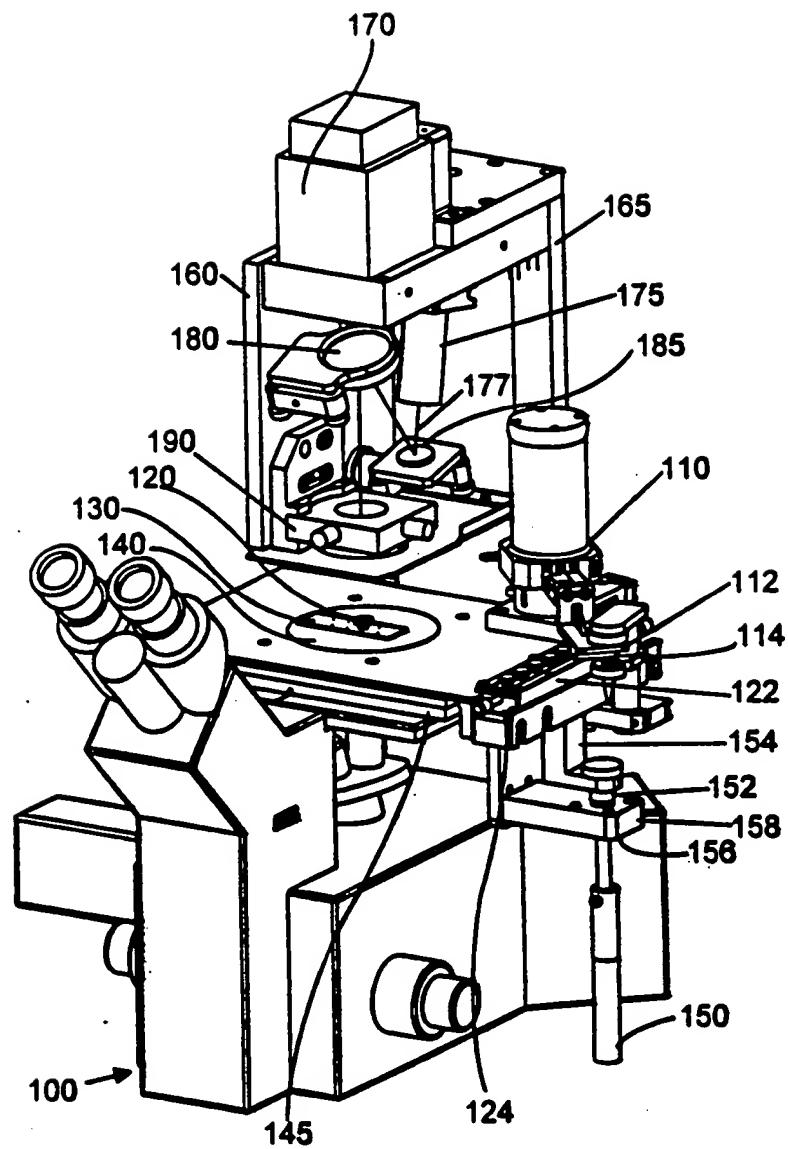
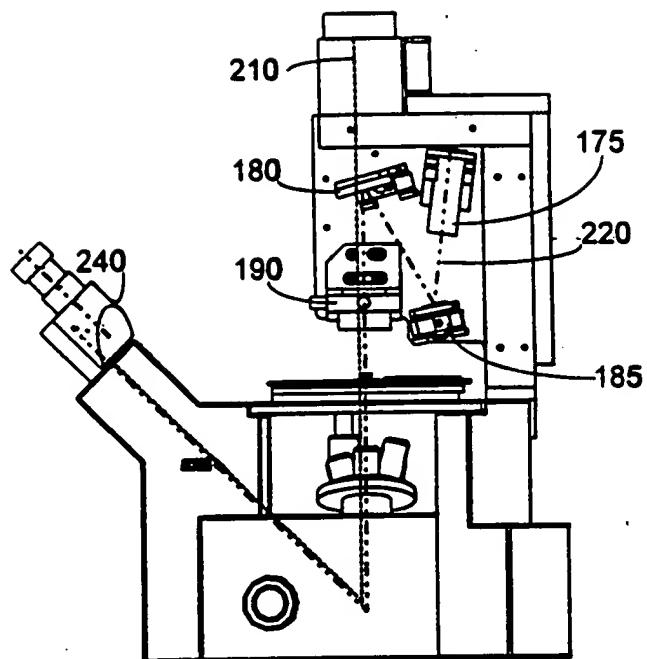
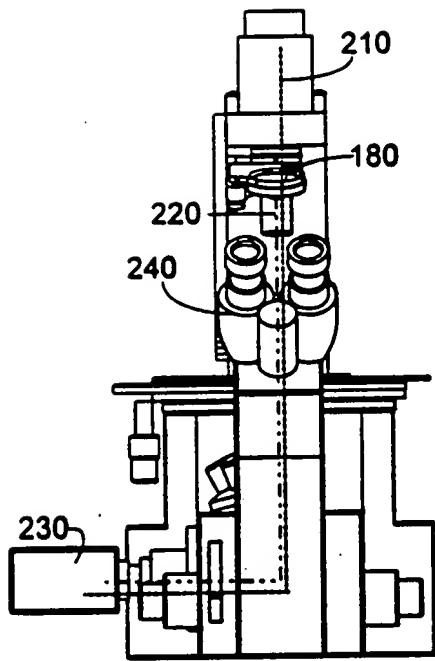


FIG. 1



— WHITE LIGHT ILLUMINATION PATH
— LASER BEAM PATH

FIG. 2A

FIG. 2B

FIG. 3

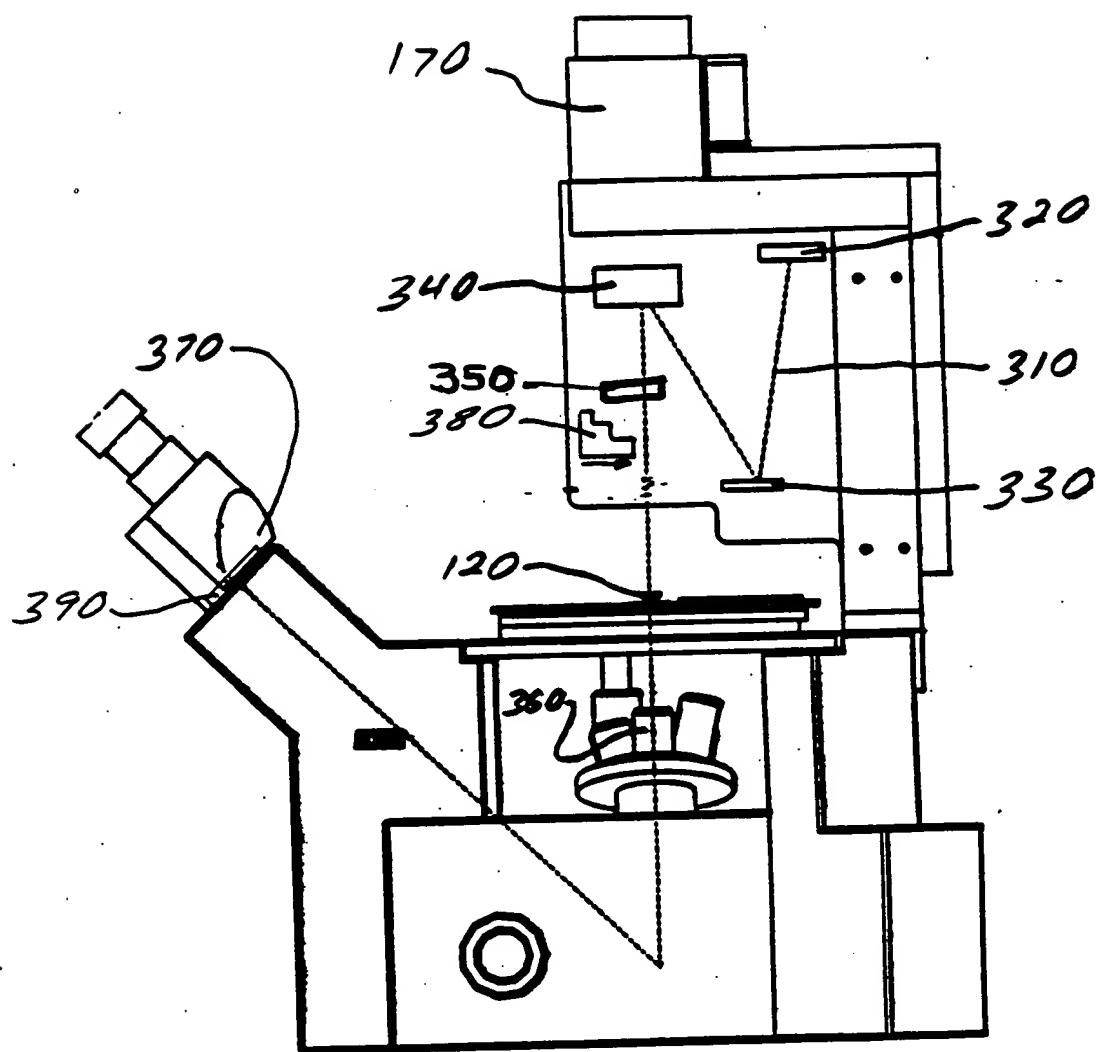
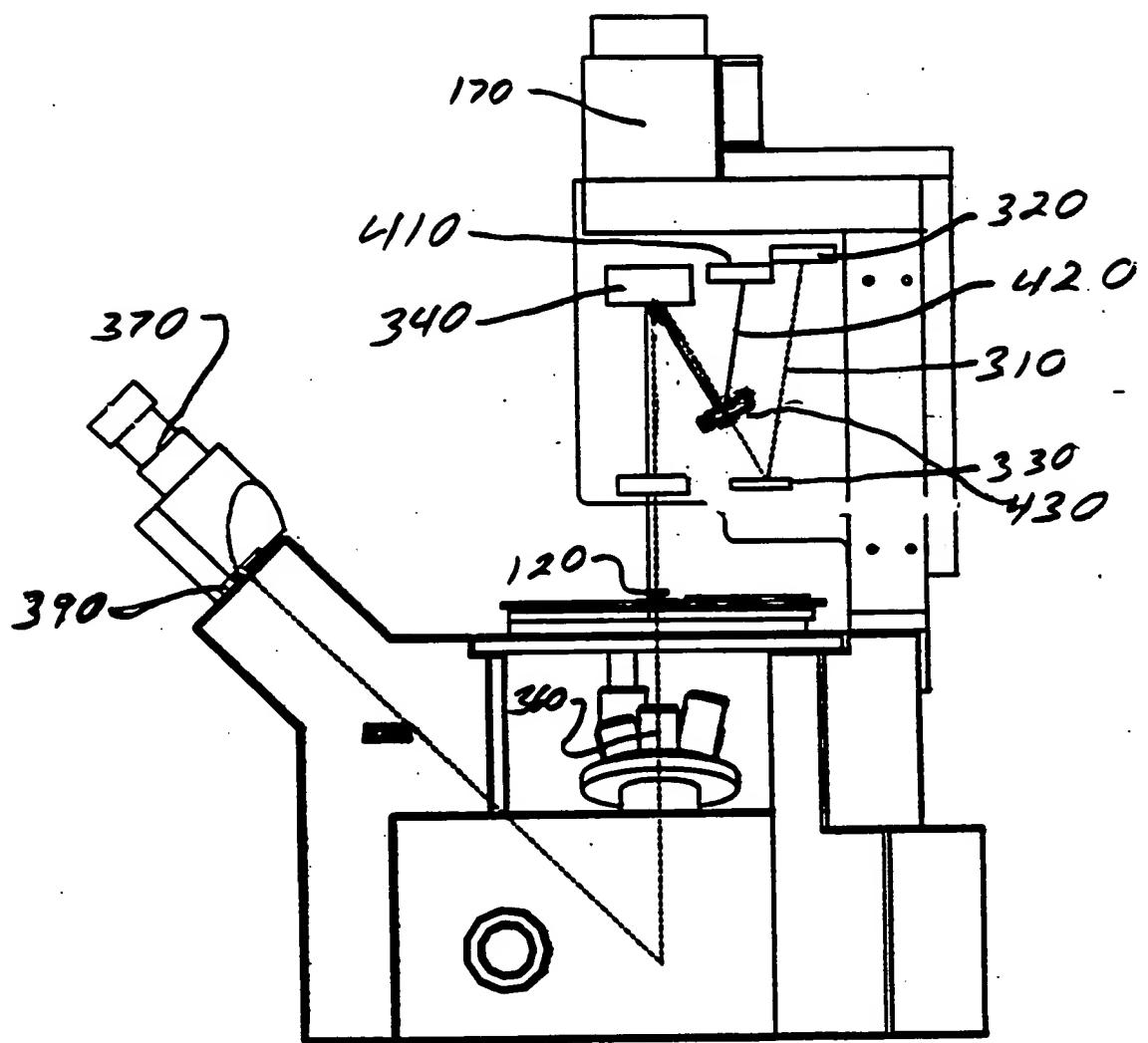


FIG. 4



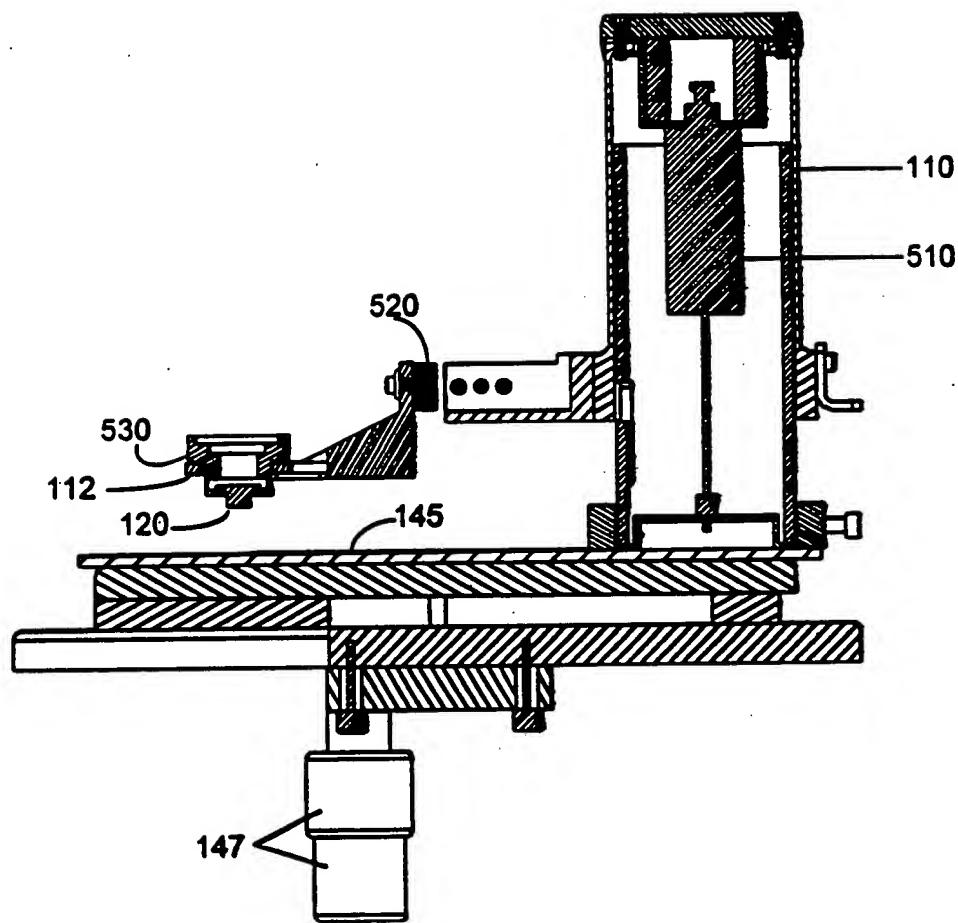


FIG. 5

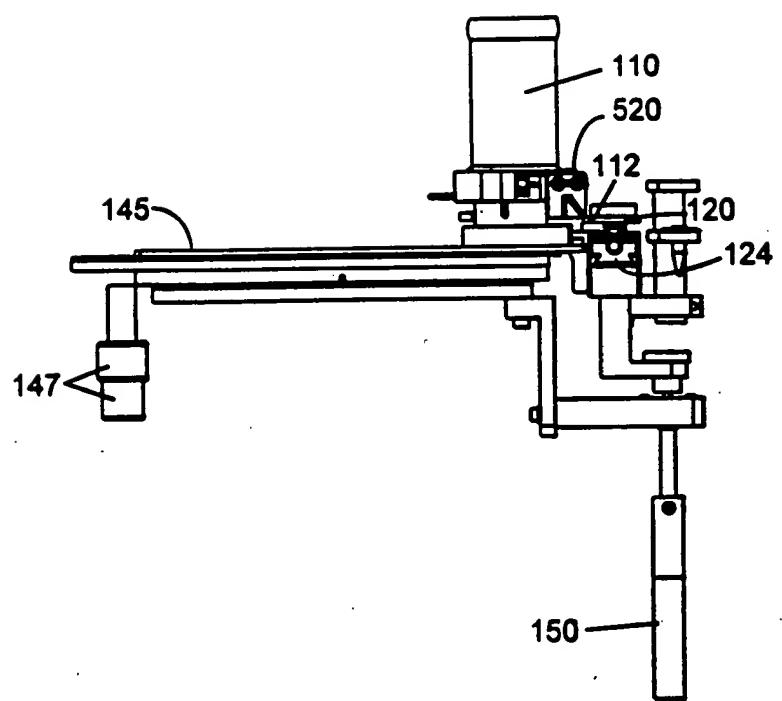


FIG. 6

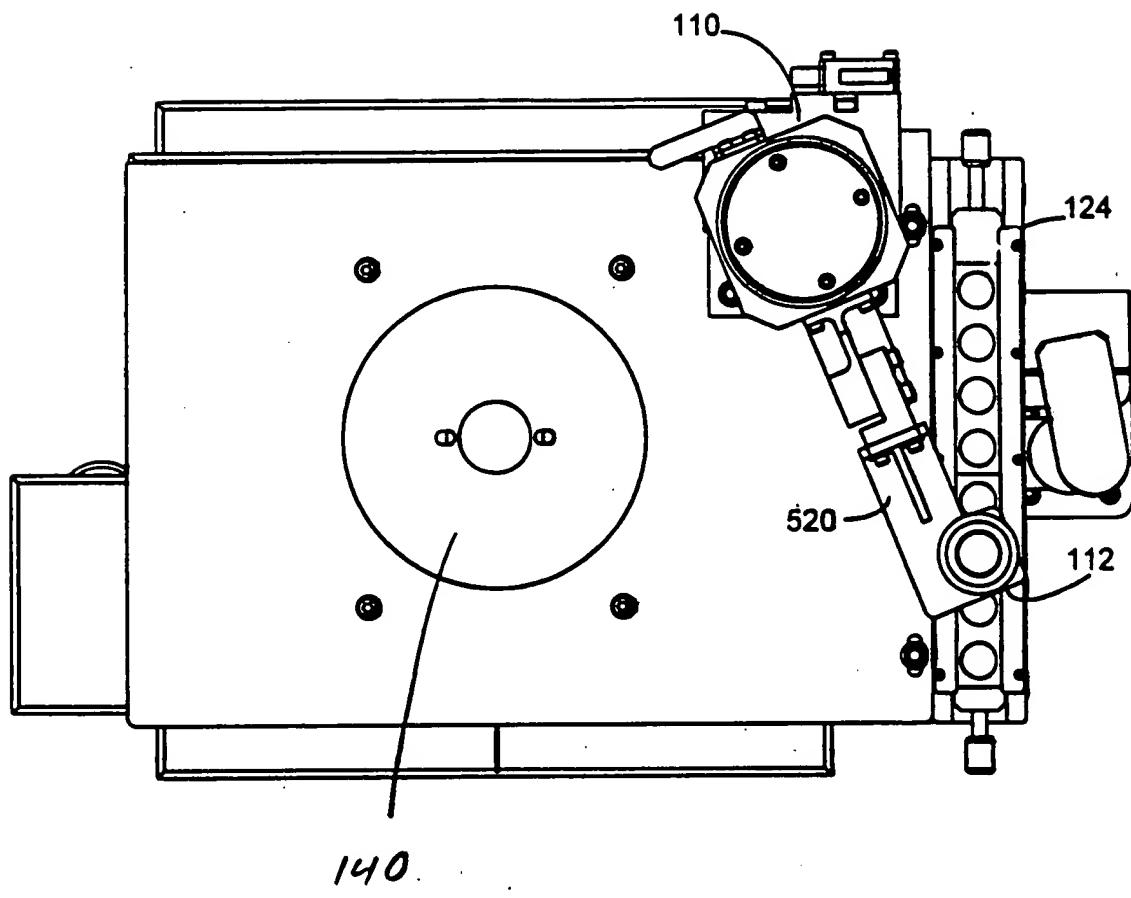


FIG. 7

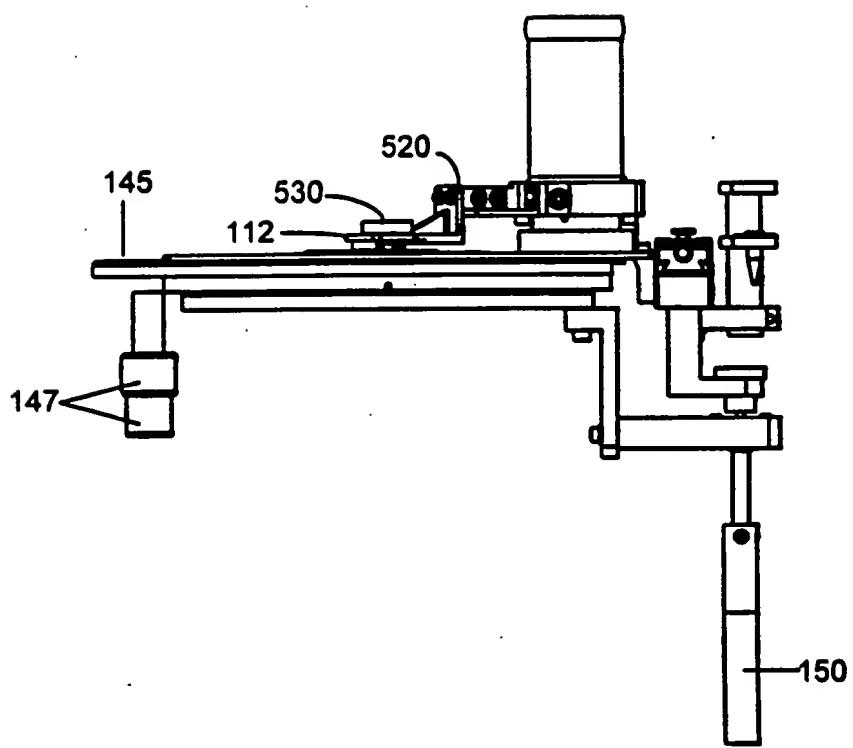


FIG. 8

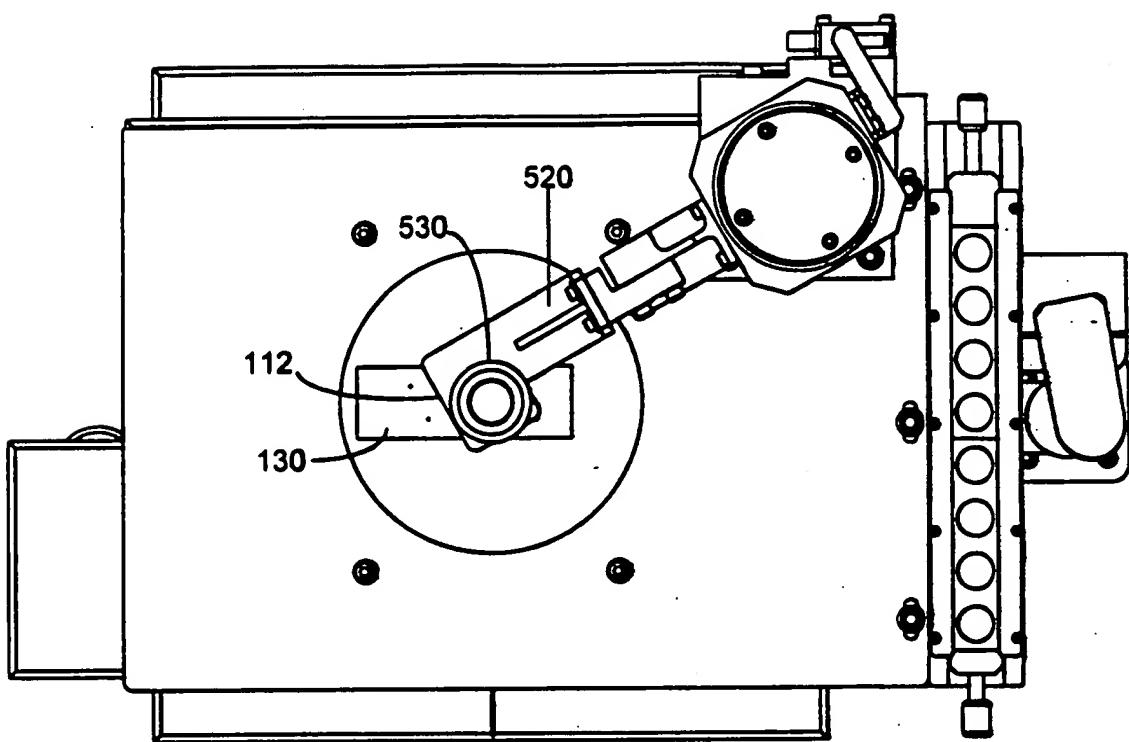


FIG. 9

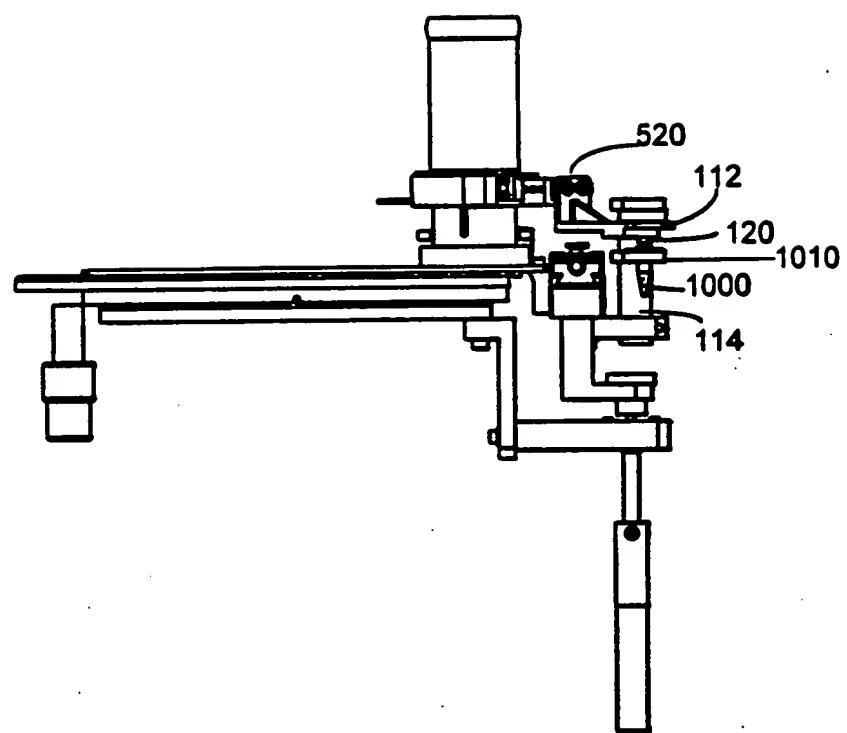


FIG. 10

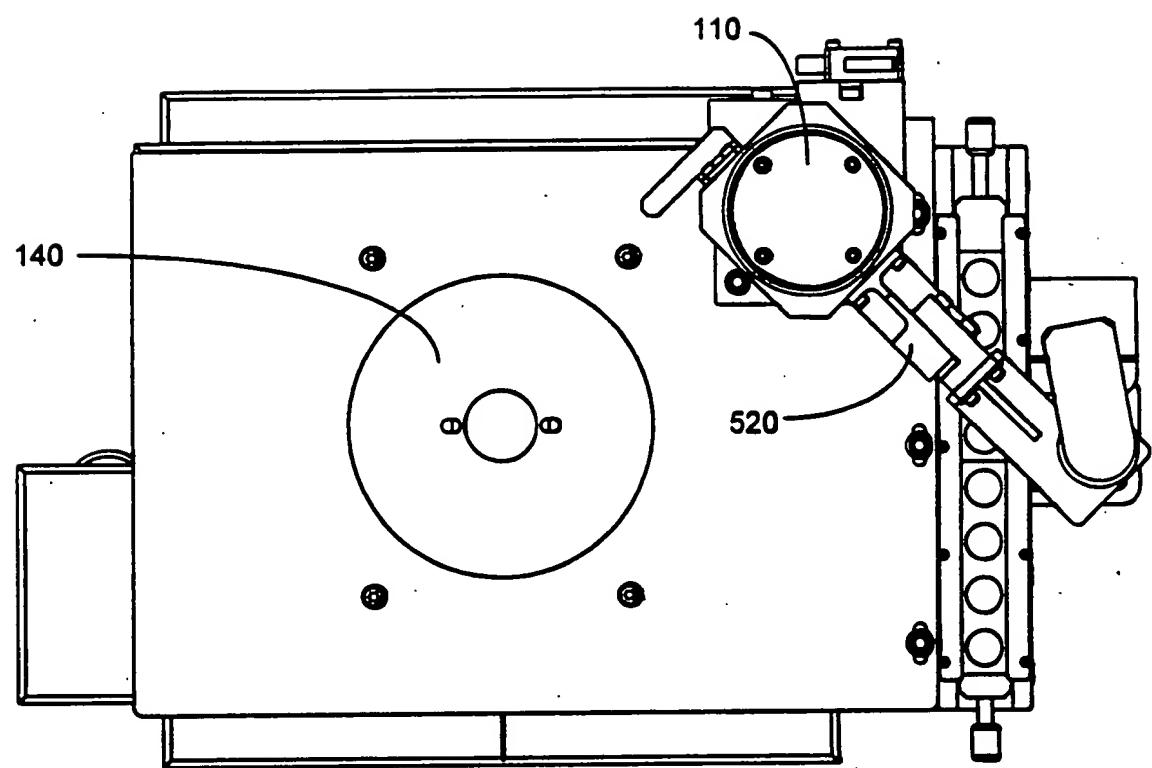


FIG. 11

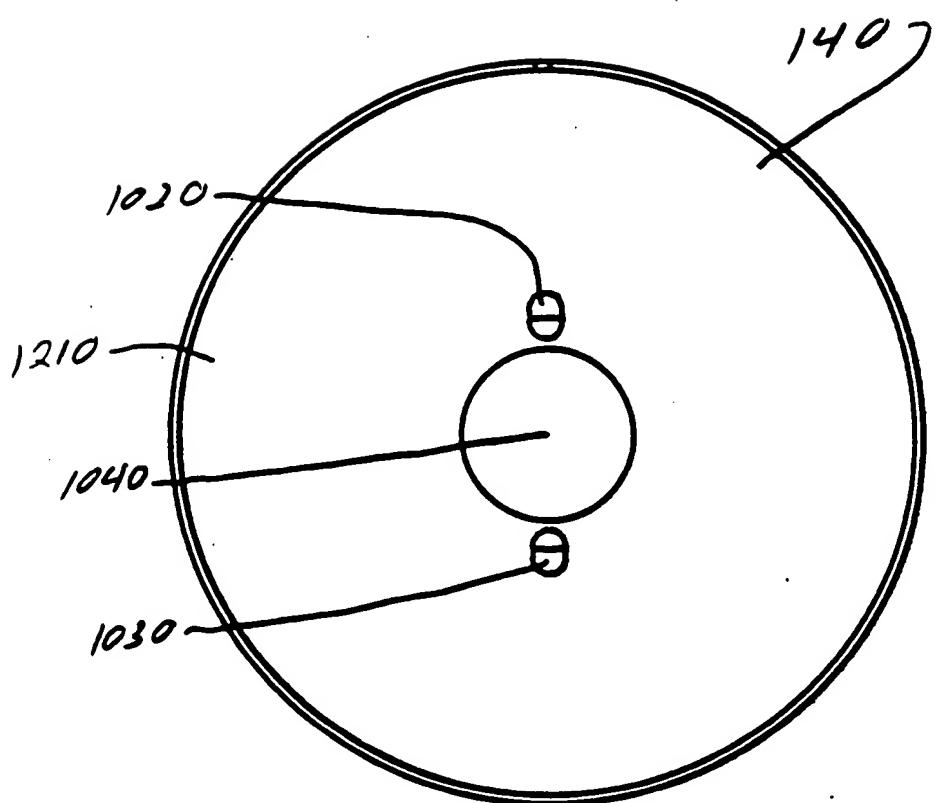
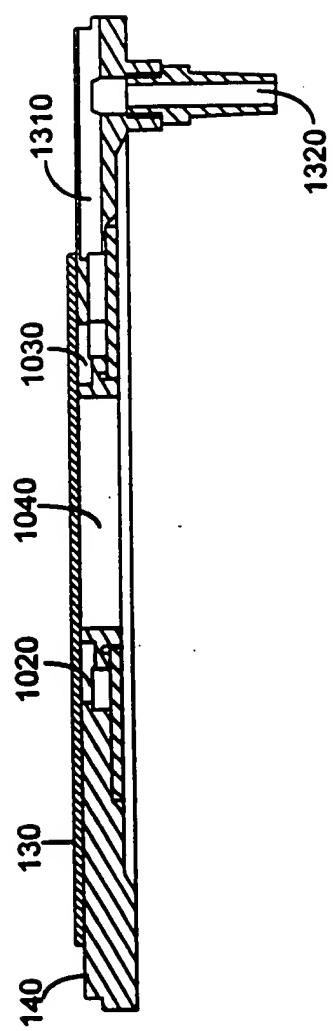


FIG. 12

FIG. 13



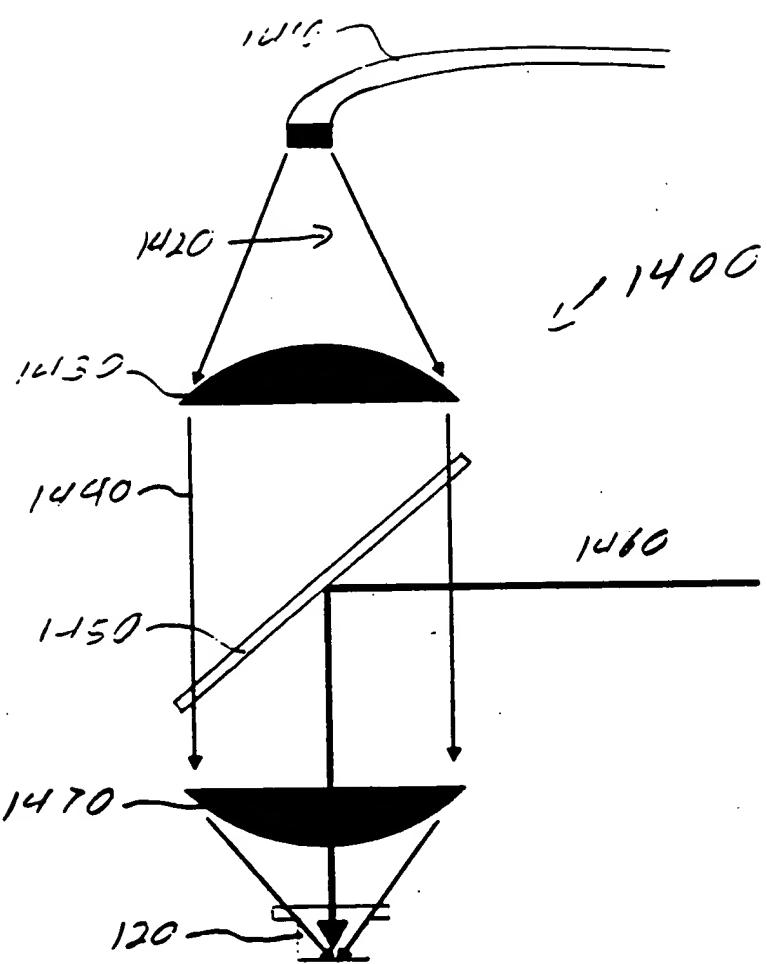


FIG 14

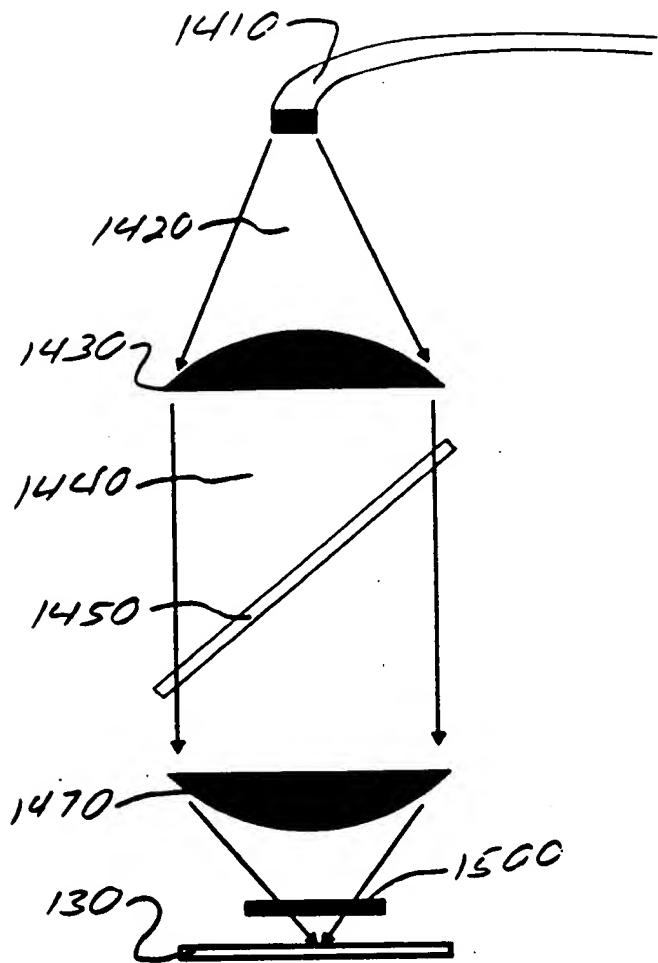


FIG 15

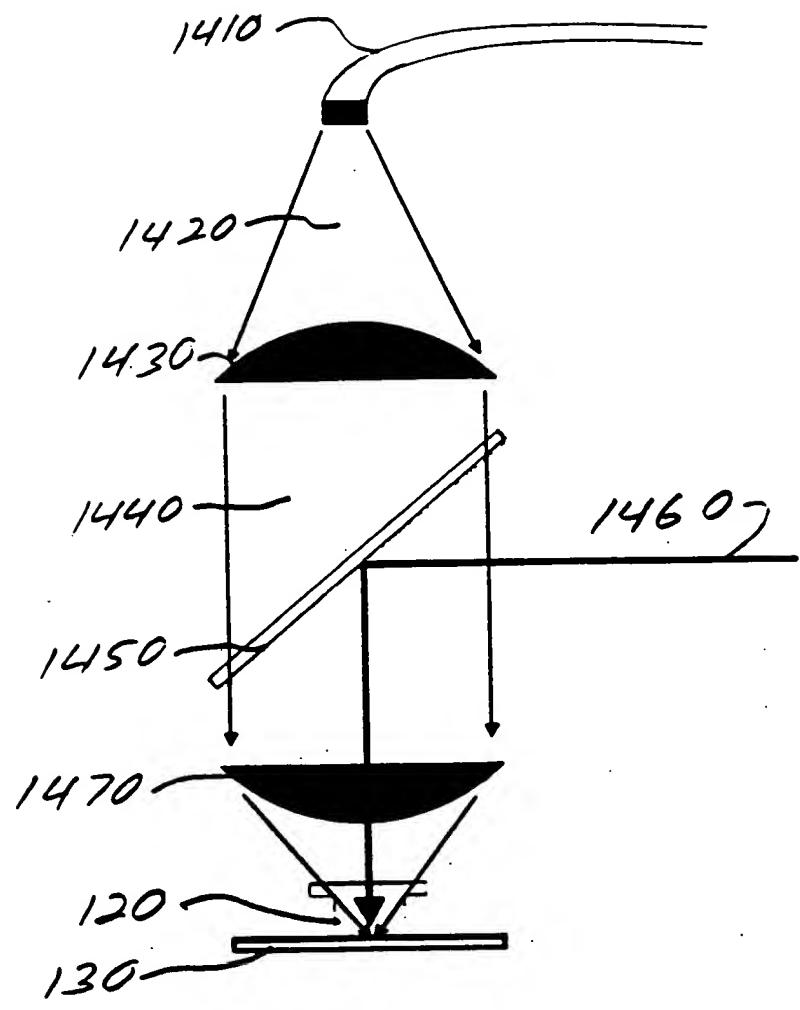


FIG. 16

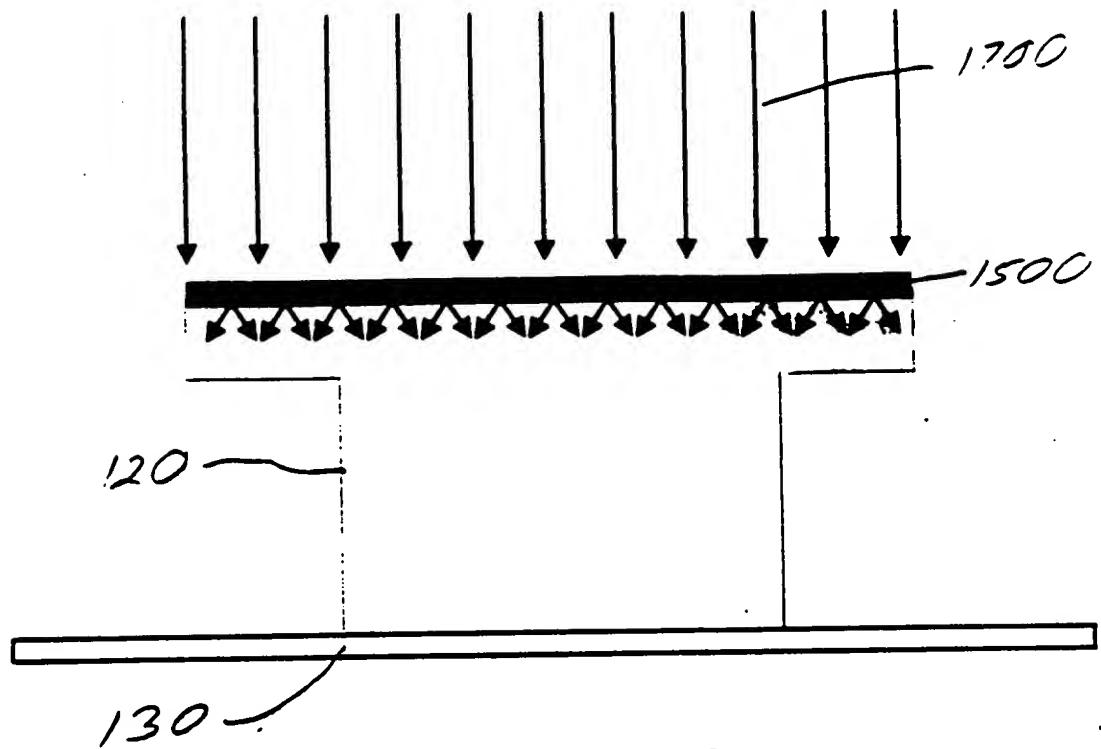
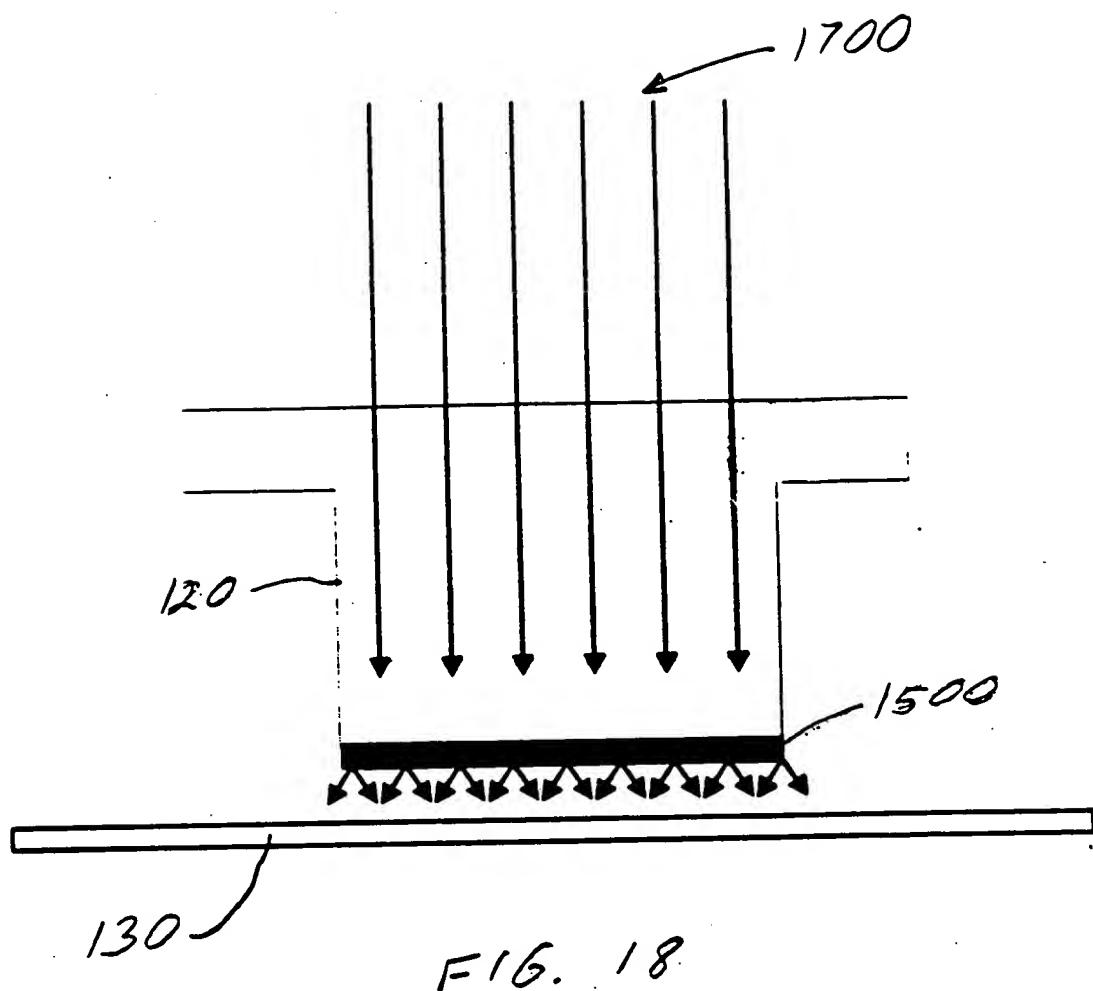


FIG. 17



09/121,635

UNITED STATES PATENT APPLICATION
ENTITLED

**LASER CAPTURE MICRODISSECTION
OPTICAL SYSTEM**

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David F. Head, and Christopher E. Todd

Citizenship: All of the United States

PATENT
Attorney Docket No. 17726-705

**LASER CAPTURE MICRODISSECTION
OPTICAL SYSTEM**

Inventors: Thomas M. Baer; Mark A. Enright
David F. Head; and Christopher E. Todd

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is, under 35 U.S.C. § 120, a continuation of U.S.
Ser. No. 09/018,452, filed February 4, 1998, now pending, which is in-turn
5 a continuation-in-part of both U.S. Ser. No. 60/060,731, filed October 1,
1997, now pending, and U.S. Ser. No. 60/037,864, filed February 7, 1997,
now abandoned, the entire contents of all which are hereby incorporated
herein by reference as if fully set forth herein.

10

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to the field of laser capture
15 microdissection (LCM). More particularly, the invention relates to inverted
microscopes that include specialized apparatus for performing LCM.
Specifically, a preferred implementation of the invention relates to an
inverted microscope that includes a cap handling subsystem, an
illumination/laser optical subsystem, a vacuum chuck subsystem, and a

manual joystick subsystem. The invention thus relates to inverted microscopes of the type that can be termed laser capture microdissection inverted microscopes.

5 **Discussion of the Related Art**

Diseases such as cancer have long been identified by examining tissue biopsies to identify unusual cells. The problem has been that there has been no satisfactory prior-art method to extract the cells of interest from 10 the surrounding tissue. Currently, investigators must attempt to manually extract, or microdissect, cells of interest either by attempting to mechanically isolate them with a manual tool or through a convoluted process of isolating and culturing the cells. Most investigators consider both approaches to be tedious, time-consuming, and inefficient.

15 A new technique has been developed which can extract a small cluster of cells from a tissue sample in a matter of seconds. The technique is called laser capture microdissection (LCM). Laser capture microdissection is a one-step technique which integrates a standard laboratory microscope with a low-energy laser and a transparent ethylene 20 vinyl acetate polymer thermoplastic film such as is used for the plastic seal in food product packaging.

In laser capture microdissection, the operator looks through a 25 microscope at a tissue biopsy section mounted on a standard glass histopathology slide, which typically contains groups of different types of cells. A thermoplastic film is placed over and in contact with the tissue biopsy section. Upon identifying a group of cells of interest within the

tissue section, the operator centers them in a target area of the microscope field and then generates a pulse from a laser such as a carbon dioxide laser having an intensity of about 50 milliwatts (mW) and a pulse duration of between about 50 to about 500 milliseconds (mS). The laser pulse causes
5 localized heating of the plastic film as it passes through it, imparting to it an adhesive property. The cells then stick to the localized adhesive area of the plastic tape directly above them, whereupon the cells are immediately extracted and ready for analysis. Because of the small diameter of the laser beam, extremely small cell clusters may be microdissected from a tissue
10 section.

By taking only these target cells directly from the tissue sample, scientists can immediately analyze the gene and enzyme activity of the target cells using other research tools. Such procedures as polymerase chain reaction amplification of DNA and RNA, and enzyme recovery from the
15 tissue sample have been demonstrated. No limitations have been reported in the ability to amplify DNA or RNA from tumor cells extracted with laser capture microdissection.

Laser capture microdissection has successfully extracted cells in all tissues in which it has been tested. These include kidney glomeruli, in situ breast carcinoma, atypical ductal hyperplasia of the breast, prostatic
20 interepithelial neoplasia, and lymphoid follicles. The direct access to cells provided by laser capture microdissection will likely lead to a revolution in the understanding of the molecular basis of cancer and other diseases, helping to lay the groundwork for earlier and more precise disease detection.

25 Another likely role for the technique is in recording the patterns of gene expression in various cell types, an emerging issue in medical research.

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For instance, the National Cancer Institute's Cancer Genome Anatomy Project (CGAP) is attempting to define the patterns of gene expression in normal, precancerous, and malignant cells. In projects such as CGAP, laser capture microdissection is a valuable tool for procuring pure cell samples from tissue samples.

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The LCM technique is generally described in the recently published article: Laser Capture Microdissection, Science, Volume 274, Number 5289, Issue 8, pp 998-1001, published in 1996, the entire contents of which are incorporated herein by reference. The purpose of the LCM technique is to provide a simple method for the procurement of selected human cells from a heterogeneous population contained on a typical histopathology biopsy slide.

15

A typical tissue biopsy sample consists of a 5 to 10 micron slice of tissue that is placed on a glass microscope slide using techniques well known in the field of pathology. This tissue slice is a cross section of the body organ that is being studied. The tissue consists of a variety of different types of cells. Often a pathologist desires to remove only a small portion of the tissue for further analysis.

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LCM employs a thermoplastic transfer film that is placed on top of the tissue sample. This film is manufactured containing organic dyes that are chosen to selectively absorb in the near infrared region of the spectrum overlapping the emission region of common AlGaAs laser diodes. When the film is exposed to the focused laser beam the exposed region is heated by the laser and melts, adhering to the tissue in the region that was exposed.

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The film is then lifted from the tissue and the selected portion of the tissue is removed with the film.

Thermoplastic transfer films such as a 100 micron thick ethyl vinyl acetate (EVA) film available from Electroseal Corporation of Pompton Lakes, New Jersey (type E540) have been used in LCM applications. The film is chosen to have a low melting point of about 90° C.

5 The thermoplastic EVA films used in LCM techniques have been doped with dyes, such as an infrared naphthalocyanine dye, available from Aldrich Chemical Company (dye number 43296-2 or 39317-7). These dyes have a strong absorption in the 800 nm region, a wavelength region that overlaps with laser emitters used to selectively melt the film. The dye is
10 mixed with the melted bulk plastic at an elevated temperature. The dyed plastic is then manufactured into a film using standard film manufacturing techniques. The dye concentration in the plastic is about 0.001 M.

15 While the films employed in LCM applications have proved satisfactory for the task, they have several drawbacks. The optical absorption of a dye impregnated film is a function of its thickness. This property of the film may be in conflict with a desire to select film thickness for other reasons.

20 The organic dyes which are used to alter the absorption characteristics of the films may have detrimental photochemistry effects in some cases. This could result in contamination of LCM samples. In addition, the organic dyes employed to date are sensitive to the wavelength of the incident laser light and thus the film must be matched to the laser employed.

SUMMARY OF THE INVENTION

There is a particular need for an instrument that is well suited for laser capture microdissection. There is also a particular need for an improved method of laser capture microdissection.

5 A first aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection method, comprising: providing a sample that is to undergo laser capture microdissection; positioning said sample within an optical axis of a laser capture microdissection instrument;

10 providing a transfer film carrier having a substrate surface and a laser capture microdissection transfer film coupled to said substrate surface; placing said laser capture microdissection transfer film in juxtaposition with said sample with a pressure sufficient to allow laser capture microdissection transfer of a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection film; and then transferring a portion of said sample to said laser capture microdissection transfer film, without forcing nonspecific transfer of a remainder of said sample to said laser capture microdissection transfer film.

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20 A second aspect of the invention is implemented in an embodiment that is based on a laser capture microdissection instrument, comprising: an inverted microscope including: an illumination/laser optical subsystem; a translation stage coupled to said illuminator/laser optical subsystem; a cap handling subsystem coupled to said translation stage; a vacuum chuck subsystem coupled to said translation stage; and a manual joystick subsystem coupled to said translation stage.

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These, and other, aspects and objects of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating preferred embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such modifications.

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BRIEF DESCRIPTION OF THE DRAWINGS

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A clear conception of the advantages and features constituting the invention, and of the components and operation of model systems provided with the invention, will become more readily apparent by referring to the exemplary, and therefore nonlimiting, embodiments illustrated in the drawings accompanying and forming a part of this specification, wherein like reference numerals (if they occur in more than one view) designate the same elements. Consequently, the claims are to be given the broadest interpretation that is consistent with the specification and the drawings. It should be noted that the features illustrated in the drawings are not necessarily drawn to scale.

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FIG. 1 illustrates a perspective view of a laser capture microdissection inverted microscope, representing an embodiment of the invention;

FIGS. 2A-2B illustrate orthographic views of the laser capture microdissection (LCM) inverted microscope shown in FIG. 1;

FIG. 3 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

5 FIG. 4 illustrates a partial cross-sectional view of an LCM inverted microscope, representing an embodiment of the invention;

FIG. 5 illustrates a cross-sectional view of a cap handling subassembly, representing an embodiment of the invention;

10 FIG. 6 illustrates an elevational view of a cap handling subassembly in a load position, representing an embodiment of the invention;

FIG. 7 illustrates a top plan view of the apparatus in the position depicted in FIG. 6;

FIG. 8 illustrates an elevational view of a cap handling subassembly in an inspect position, representing an embodiment of the invention;

15 FIG. 9 illustrates a top plan view of the apparatus in the position depicted in FIG. 8;

FIG. 10 illustrates an elevational view of a cap handling subassembly in an unload position, representing an embodiment of the invention;

20 FIG. 11 illustrates a top plan view of the apparatus in the position depicted in FIG. 10;

FIG. 12 illustrates a top plan view of a vacuum chuck, representing an embodiment of the invention;

25 FIG. 13 illustrates a cross-sectional view of a vacuum chuck, representing an embodiment of the invention;

FIG. 14 illustrates a schematic diagram of a combined illumination light/laser beam delivery system, representing an embodiment of the invention;

5 FIG. 15 illustrates a schematic view of a combined illumination/laser beam delivery system with a diffuser in place, representing an embodiment of the invention;

10 FIG. 16 illustrates a schematic view of a combined illumination/laser beam delivery system with a cap in place, representing an embodiment of the invention;

15 FIG. 17 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention; and

20 FIG. 18 illustrates a schematic view of an integrated cap/diffuser, representing an embodiment of the invention.

15 DESCRIPTION OF PREFERRED EMBODIMENTS

The invention and the various features and advantageous details thereof are explained more fully with reference to the nonlimiting embodiments that are illustrated in the accompanying drawings and detailed 20 in the following description. Descriptions of well known components and processing techniques are omitted so as not to unnecessarily obscure the invention in detail.

25 The entire contents of U.S. Ser. No. 60/037,864, filed February 7, 1997 entitled "Laser Capture Microdissection Device," (Docket No. ARCT-002); U.S. Ser. No. 08/797,026, filed February 7, 1997; U.S. Ser. No. 08/800,882, filed February 14, 1997; U.S. Ser. No. 60/060,731, filed

October 1, 1997; and U.S. Ser. No. 60/060,732, filed October 1, 1997 are hereby expressly incorporated by reference into the present application as if fully set forth herein.

Turning to FIG. 1, a perspective view of an inverted microscope 100 for laser capture microdissection (LCM) is depicted. The inverted microscope 100 includes a variety of subsystems, particularly adapted for the LCM technique which combine to provide synergistic and unexpectedly good results. In alternative embodiments, the microscope does not need to be an inverted microscope.

A cap handling mechanic subassembly 110 provides structure for picking a microcentrifuge tube cap 120 from a supply 122 and placing the microcentrifuge tube cap 120 on top of a sample that is to undergo LCM. In the depicted embodiment, the microcentrifuge tube cap 120 is a cylindrical symmetric plastic cap and the supply 122 includes eight of the consumables on a dovetail slide 124. In the depicted embodiment, there is a laser capture microdissection transfer film coupled to the bottom of the microcentrifuge tube cap 120. The cap handling mechanic subassembly 110 is depicted in one of several possible positions wherein a working end 112 of the cap handling mechanic subassembly 110 is positioned in a vial capping station 114. The movement of the cap handling mechanic subassembly 110 will be described in more detail below.

A glass slide 130 upon which the sample to be microdissected is located and upon which the microcentrifuge tube cap 120 is placed, is located in the primary optical axis of the inverted microscope 100. In alternative embodiments, the slide that supports the sample can be made of other substantially transparent materials, for example, plastics such as

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polycarbonate. The glass slide 130 is supported and held in place by a vacuum chuck 140. The vacuum chuck 140 is a substantially flat surface that engages the glass slide 130 through a manifold (not shown) so as to hold the glass slide 130 in place while the microcentrifuge tube cap 120 is picked and placed and while a translation stage 145 is manipulated in an X-Y plane. In alternative embodiments, the translation stage can be configured so as to have the capability of being moved along a Z axis.

10

The translation stage 145 can be manipulated using a pair of rotary controls (not shown in FIG. 1). In addition, the translation stage 145 can be manipulated using a joystick 150. The joystick 150 is connected to the translation stage 145 through a spherical mounting 152 and a bracket 154. The joystick 150 includes a second spherical mounting 156 within a static bracket 158. The joystick provides simultaneous X and Y movement. Further, this simultaneous movement can be effected with in a single handed manner. The acquisition of samples is thus made quicker.

15

Mechanical leverage is provided by the fact that the length between the spherical mounting 152 and the second spherical mounting 156 is less than the length between the second spherical mounting 156 and the bottom end of the joystick 150. This leverage ratio is not needed for multiplication of force, but for the reduction in scalar movement. This ratio should be less than 1/5, preferably approximately 1/7. This ratio can be adjusted to provide the optimal resolution needed in terms of sample movement as a function of operator hand movement.

20

In addition, the joystick provides tactile feedback not available with electronic controls or geared linkages. The joystick 150 permits simultaneous movement of the translation stage 145 in two directions (X

and Y) as a function of a single vector movement of an operator's hand. This important feature provides an unexpected result in that the speed with which features to be microdissected can be positioned in the principal optical axis of the inverted microscope 100 is significantly increased.

5 Still referring to FIG. 1, the inverted microscope 100 includes an LCM optical train 160. The LCM optical train 160 is mounted on an illumination arm 165. A white light illuminator 170 is also mounted on the illumination arm 165. White light from the illuminator 170 passes downward toward the microcentrifuge tube cap 120 through a dichroic mirror 180 and a focusing lens 190. A laser diode 175 with collimating optics emits a beam 177 that is reflected by a beam steering mirror 185. After the beam 177 is reflected by the beam steering mirror 185 it is incident upon the dichroic mirror 180. The dichroic mirror 180 is a dichroic that reflects the beam 170 downward through the focusing lens 190 toward the microcentrifuge tube cap 120. Simultaneously, the dichroic mirror 180 allows white light from the illuminator 170 to pass directly down through the focusing lens 190 toward the microcentrifuge tube cap 120. Thus, the beam 177 and the white light illumination are superimposed. The focusing lens 190 also adjusts the beam spot size.

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20 Turning now to FIGS. 2A-2B, two orthographic views of the apparatus depicted in FIG. 1 are illustrated. A white light illumination path 210 and a laser beam path 220 can be seen in both FIGS. 2A and 2B. It can be appreciated from FIG. 2A that both of the paths include delivery of optical information to an image acquisition system 230. Similarly, it can be appreciated from FIG. 2B that the illumination beam path includes delivery

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of optical information to a binocular set 240. In alternative embodiments, the eyepiece assembly (i.e., ocular) can include a monocular.

Turning to FIG. 3, a block schematic diagram of an optical train according to the invention is depicted. A laser beam path 310 begins at a film activation laser 320. The laser beam path 310 is then reflected by a mirror 330. The laser beam path 310 is then reflected by a dichroic mirror 340. The laser beam path 310 is then focused by a lens 350. The lens 350 can optionally be associated with structure for changing the beam diameter such as, for example, a variable aperture. The laser beam path 310 then passes downward toward the microcentrifuge tube cap 120. The laser beam path 310 then passes through an objective lens 360 and is then reflected. A cut-off filter 390 is installed in the ocular 370. The cut-off filter 390 can reflect and/or absorb the energy from the laser beam.

The position of the laser beam path 310 with respect to the portion of the sample that is to be acquired by the microcentrifuge tube cap 120 can be seen by an operator via the image acquisition system 230 (not shown in FIG. 3), which can include a camera. In idle mode, the laser beam path 310 provides a visible low amplitude signal that can be detected via the acquisition system 230. In pulse mode, the laser beam path 310 delivers energy to the microcentrifuge tube cap 120 and the optical characteristics of the cut-off filter 390 attenuate the laser beam path 310 sufficiently so that substantially none of the energy from the laser beam exits through ocular 370.

Suitable laser pulse widths are from 0 to approximately 1 second, preferably from 0 to approximately 100 milliseconds, more preferably approximately 50 milliseconds. In a preferred embodiment the wavelength

of the laser is 810 nannometers. In a preferred embodiment the spot size of
the laser at the EVA material located on microcentrifuge tube cap 120 is
variable from 0.1 to 100 microns, preferably from 1 to 60 microns, more
preferably from 5 to 30 microns. These ranges are relatively preferred when
5 designing the optical subsystem. From the standpoint of the clinical
operator, the widest spot size range is the most versatile. A lower end point
in the spot size range on the order of 5 microns is useful for transferring
single cells.

Suitable lasers can be selected from a wide power range. For
10 example, a 100 watt laser can be used. On the other hand, a 50 mW laser
can be used. The laser can be connected to the rest of the optical subsystem
with a fiber optical coupling. Smaller spot sizes are obtainable using
diffraction limited laser diodes and/or single mode fiber optics. Single
mode fiber allows a diffraction limited beam.

15 While the laser diode can be run in a standard mode such as TEM₀₀,
other intensity profiles can be used for different types of applications.
Further, the beam diameter could be changed with a stepped lens instead of
lens 350.

20 Changing the beam diameter permits the size of the portion of the
sample that is acquired to be adjusted. Given a tightly focused initial
condition, the beam size can be increased by defocusing. Given a defocused
initial condition, the beam size can be decreased by focusing. The change in
focus can be in fixed amounts. The change in focus can be obtained by
means of indents on a movable lens mounting and/or by means of optical
25 glass steps. In any event, increasing/decreasing the optical path length is the
effect that is needed to alter the focus of the beam, thereby altering the spot

size. For example, inserting a stepped glass prism 380 into the beam so the beam strikes one step tread will change the optical path length and alter the spot size.

Turning now to FIG. 4, a schematic block diagram of another embodiment of an instrument according to the invention is depicted. In this embodiment, a light source 410 (e.g., fluorescence laser), emits a specific wavelength or wavelength range. The specific wavelength or wavelength range of a beam 420 emitted by the light source 410 is chosen, or filtered, to excite a fluorescent system (e.g., chemical markers and optical filtering techniques that are known in the industry) that is incorporated in or applied to the sample to be microdissected. The frequency of a beam 420 emitted by the fluorescence laser 410 can be tuned. The sample includes at least one member selected from the group consisting of chromophores and fluorescent dyes (synthetic or organic), and, the process of operating the instrument includes identifying at least a portion of said sample with light that excites the at least one member, before the step of transferring said portion of said sample to said laser capture microdissection transfer film.

Still referring to FIG. 4, the beam 420 is reflected by a mirror 430. The beam 420 is then reflected by the dichroic mirror 340. In this way the beam 420 can be made coincident with both the laser beam path 310 and the white light from illuminator 170. It should be noted that the beam 420 and the laser beam path 310 are shown in a spaced-apart configuration for clarity only. The beam 420 and the laser beam path 310 can be coaxial. Fluorescence emitted by the sample beneath the microcentrifuge tube cap 120 then travels through the objective lens 360 to be viewed by the operator through ocular 370.

Turning now to FIG. 5, a cross-sectional view of the cap handling mechanic subassembly 110 is depicted. The cap handling mechanic subassembly 110 includes a dampener 510. The dampener 510 is a structure for damping vertical motion of the cap handling mechanic subassembly 110.

5 The dampener 510 is adapted to lower the microcentrifuge tube cap 120 down towards the translation stage in a reproducible manner. The dampener 510 can be an air dampener (e.g., pneumatic tube) or liquid dampener (e.g., hydraulic tube) or any other dynamic structure capable of retarding the vertical motion of the subassembly 110 so as not to generate an impulse. As

10 the microcentrifuge tube cap 120 contacts the slide on which the sample rests (not shown), the working end 112 of an arm 520 that is coupled to the dampener 510 continues downward at a reproducible rate. Therefore, the top of the microcentrifuge tube cap 120 rises relative to the bottom of a weight 530. It can be appreciated that the cap 120 contacts the slide, before

15 the weight 530 contacts the cap 120. In this way, the microcentrifuge tube cap 120 undergoes a self-leveling step before it is contacted and pressed against the slide by weight 530. As the weight 530 contacts the microcentrifuge tube cap 120 the working end 112 of arm 520 continues along its downward path. Therefore, the application of the weight 530 to

20 microcentrifuge tube cap 120 is also a self-leveling step. By controlling the mass of weight 530, the force per unit area between the bottom of the microcentrifuge tube cap 120 and the slide can be controlled. After the sample on the slide has undergone LCM, the arm 520 can be raised. By raising the arm, the weight 530 is first picked off the microcentrifuge tube

25 cap 120 and then the microcentrifuge tube cap 120 is picked up off of the

slide. The dampener within the mechanism acts as a dash pot to control the velocity of the pickup arm.

The position of the translation stage is independent relative to the position of the cap handling mechanic subassembly 110. These relative positions can be controlled by the pair of rotary controls 147. It is to be noted that the pair of rotary controls 147 are depicted with their axes parallel to the axis of the microcentrifuge tube cap 120 in FIG. 5. However, the pair of rotary controls 147 can be configured in any orientation through the use of mechanical linkages such as gears.

Turning now to FIG. 6, the cap handling mechanic subassembly 110 is depicted in a load position. In the load position, the working end 112 of the arm 520 is located directly over the dovetail slide 124. In this position, the working end 112 grasps a microcentrifuge tube cap 120. After grasping the microcentrifuge tube cap 120, the arm 520 is raised, thereby picking the microcentrifuge tube cap 120 up.

Turning now to FIG. 7, a top plan view of the cap handling mechanic subassembly 110 in the load position can be seen. Before the arm 520 is swung into the load position, a fresh microcentrifuge tube cap is located beneath the axis of the working end 112. After the arm 520 is swung clockwise toward the vacuum chuck 140, the caps on dovetail slide 124 will be advanced so as to position a fresh microcentrifuge tube cap in place for the next cycle.

Turning now to FIG. 8, the cap handling mechanic subassembly 110 is depicted in an inspect position. When positioned in the inspect position, the working end 112 of the arm 520 is located coincident with the principal optical axis of the instrument. This is the position in which the arm 520 is

lowered to permit first the self-leveling of the microcentrifuge tube cap 120 and then the self-leveling of the weight 530 on top of the microcentrifuge tube cap 120. After LCM, the arm 520 is raised in this position to put the weight 530 off the microcentrifuge tube cap 120 and then the

5 microcentrifuge tube cap 120 off the slide (not shown).

The weight 530 is a free floating weight so that when it is set on top of the cap, the cap and weight are free to settle. The free floating weight permits the even application of pressure. For example, a weight of 30 grams can be used in the case where the total surface area of the laser capture

10 microdissection transfer film is approximately 0.26 square inches.

Referring now to FIG. 9, a top plan view of the cap handling mechanic subassembly 110 in the inspect position is depicted. It can be appreciated from this view that the working end 112 of the arm 520 is located above the glass slide 130.

15 Turning now to FIG. 10, the cap handling mechanic subassembly 110 is depicted in an unload position. In the unload position, the working end 112 of the arm 520 and the cap 120 (aka consumable) with the LCM attached tissue are all located above the vial capping station 114. After being positioned on axis with the vial capping station 114, the

20 microcentrifuge tube cap 120 is lowered directly down onto, and into, an analysis container 1000. After the microcentrifuge tube cap 120 is inserted into the analysis container 1000, the working end 112 of the arm 520 is raised up. The working end 112 of the arm 520 is then rotated in a clockwise direction until it is above a fresh consumable (corresponding to

25 the position depicted in FIGS. 6-7).

Turning now to FIG. 11, a top plan view of the cap handling mechanic subassembly 110 in the unload position is depicted. In this position, the arm 520 is positioned away from the vacuum chuck 140. The analysis container 1000 (not visible in FIG. 11) is pushed upward so as to engage the microcentrifuge tube cap 120 (not visible in FIG. 11). The resultant sealed analysis container 1000 is then allowed to free fall back into a supporting bracket 1010 (see FIG. 10). The sealed analysis container 1000 together with the microcentrifuge tube cap 120 can then be taken from the bracket 1010 either manually or automatically.

Turning now to FIG. 12, a top plan view of the vacuum chuck 140 is depicted. A top surface 1210 of the vacuum chuck 140 includes a first manifold hole 1020 and a second manifold hole 1030. In alternative embodiments, there can be any number of manifold holes. The vacuum chuck 140 includes a beam path hole 1040. When the instrument is in operation, the glass slide (not shown), or other sample holder, is placed over the beam path hole 1040 and the manifold holes 1020-1030. After the glass slide is placed in position, a vacuum is pulled through a manifold connected to the holes 1020-1030, thereby holding the glass slide in position over the beam path hole 1040. Although a medium or even a high vacuum can be applied, a low vacuum is sufficient to hold the glass slide in place during the LCM process. A sufficient vacuum can even be generated with an inexpensive aquarium pump run in reverse.

The holding force exerted on the glass slide 130 is a function of the applied vacuum, the size and shape of the manifold holes 1020-1030 and the spacing between the top surface of the translation stage and the bottom surface of the glass slide 130. The spacing between the translation stage and

the glass slide 130 is a function of the flatness of the surfaces and the elasticity of the corresponding structures.

5 The level of vacuum is adjusted so that the glass slide 130, or other sample carrier, can be translated with regard to the translation stage. This translation capability is present when the vacuum is off and when the vacuum is on.

10 There is some leakage around the perimeter of the glass slide 130 which modulates the force holding the glass slide 130 in place. Accordingly, a moderate force (e.g., 5 pounds) applied to the edge of the glass slide is sufficient to cause movement of the glass slide 130 with regard to the translation stage when the vacuum is engaged.

15 Turning now to FIG. 13, a cross section of the vacuum chuck is depicted with a glass slide 130 in place. The vacuum that holds the glass slide 130 in place is pulled through conduit 1320. The conduit 1320 is connected to a circular manifold 1310. The circular manifold 1310 is coupled with the manifold holes 1020-1030.

20 It can be appreciated from FIG. 13 that there are no pins, or other structures, that project above the top surface of the vacuum chuck 140. This permits the glass slide 130 to be moved in any direction parallel with the top surface without constraint.

25 Turning now to FIG. 14, a very high numerical aperture illuminator 1400 for an LCM device is depicted. The illuminator 1400 provides a large working distance. A fiber optic 1410 provides a source of white light illumination. The diverging beam 1420 from the fiber optic 1410 can have a numerical aperture of approximately 0.4. A collimator lens 1430 collimates the light from the fiber optic 1410. The collimator lens 1430 can

be an aspheric lens (e.g., a Melles Griot (01 LAG 025) aspheric-like lens). A collimated beam 1440 from the collimator lens 1430 then passes though a beam splitter 1450. The beam splitter 1450 permits the injection of a laser beam 1460. After reflection by the beam splitter 1450, the laser beam 1460 is coaxial with the white light illumination. Both types of light then reach a condenser lens 1470. Condenser lens 1470 can be a Melles Griot (01 LAG 010) or (01 LAG 010) or other similar aspheric-like lens. The condensed coaxial beams are then incident upon and pass through the microcentrifuge tube cap 120. The focusing beam that results from the condenser lens 1470 can have a numerical aperture of approximately 0.8. This can be characterized as a focusing beam. The microcentrifuge tube cap 120 is located on top of a slide with cells to be sampled (not shown).

Turning now to FIG. 15, another embodiment of the high numerical aperture illuminator is depicted. In this embodiment, a diffuser 1500 is located beneath the condenser lens 1470 at above the glass slide 130 that contains the cells to be sampled.

More generally, any suitable scattering media can be used to provide the functions of the diffuser 1500. Providing such a scattering media near the tissue to scatter the light results in dramatically improved illumination of the sample and much better visualization. A scattering media of this type eliminates the need for refractive index matching of the sample. Such a scattering media can allow visualization of the cell nucleus and other subcellular structures that would normally be obscured by normal illumination techniques.

The scattering media can be a diffuser material. A diffuser material that is suitable for use as the scattering media is milk or opal glass which is

a very dense, fine diffuser material. For instance, a suitable milk glass is available from Edmund Scientific as Part No. P43,717. Standard laser printer/photocopier paper can even be used as the scattering media. Other types of transparent scattering media can be used, such as, for example, 5 frosted glass, a lenticular sheet, a volume diffuser, and/or a surface diffuser. In any event, the scattering media should be a material that aggressively scatters the illumination light. A single sheet of typical ground glass is generally inadequate and needs to be combined in multiple layers as a serial stack of three or four sheets of ground glass to diffuse the illumination light 10 sufficiently.

Turning now to FIG. 16, after the diffuser 1500 is replaced with a microcentrifuge tube cap 120, the desired cells can be located using the image acquired during the step represented in FIG. 15. Then, the laser beam 1460 can be introduced, reflected off the beam splitter 1450 and directed 15 into the microcentrifuge tube cap 120 so as to acquire the desired sample.

The purpose of the illuminator design is to provide a very high numerical aperture illuminator for an LCM device. Such an LCM device requires a large working distance. While an illuminator that uses a 40X objective with 0.8 numerical aperture may seem to give better visualization, 20 this design has problems since the working distance for the 40X objective is very small, (e.g., less than 1 millimeter). Thus it is critical for a design that uses a thick dome carrier to have an illumination design with a much longer working distance. A thick dome carrier is a sample carrier whose top and bottom are spaced apart more than a small distance. This is important 25 because the sample is adjacent the bottom of the sample carrier and the

objective cannot move closer to the sample than the top of the sample carrier.

5 The focusing lens 190 can be replaced with a Melles Griot aspheric condenser lens such as a 01 LAG 010. Such a lens has a numerical aperture of about 0.75 and a working distance of about 25 millimeters. Such a lens is not corrected for chromatic aberrations like the 40X objective. Experiments done using a spherical lens as a condenser gave good improvement in visualization. This spherical lens clearly did not have the corrections for aberrations that are built into the 40X objective.

10 The laser beam can be focused through this condenser lens like the focusing lens 190. This condenser lens has roughly one-half the focal length of the current lens so the laser beam will be focused down to roughly 15 microns.

15 In an alternative embodiment the design could use a compound lens like the lens in a barcode scanner. Such a compound lens would have a central region for the laser and a surrounding region that would act as a high numerical aperture with regard to the white light illumination.

20 Turning now to FIG. 17, in one embodiment the diffuser 1500 can be located adjacent to the microcentrifuge tube cap 120. In this embodiment the microcentrifuge tube cap 120 is located just above the glass slide 130. Collimated light 1700 is incident upon the diffuser 1500. The diffuser 1500 causes the collimated light to enter into and pass through the cap at an infinite variety of angles. In this way, shadows are reduced and the quality of the imagery is improved.

25 The diffuser 1500 can be a volumetric diffuser or a surface diffuser. In the case of a volumetric diffuser, the diffuser 1500 can be frosted glass, a

speckle based holographic diffuser or even a piece of paper. In the case of a surface diffuser, the diffuser 1500 can be a lenticular sheet, a speckle based holographic surface diffuser or any other suitable topological surface.

Turning now to FIG. 18, the diffuser 1500 in this embodiment is located adjacent to the bottom of the microcentrifuge tube cap 120. The collimated light 1700 passes through the microcentrifuge tube cap 120 and is incident upon the diffuser 1500. As the previously collimated light emerges from the diffuser 1500 it is scattered into a wide range of angles. In this embodiment, the diffuser 1500 is spaced apart from the glass slide 130.

The scattering media (e.g., diffuser 1500) can be directly or indirectly connected to the transfer film carrier and/or the LCM transfer film. Alternatively, the scattering media can be formed on a surface of, or the interior of, the transfer film carrier and/or the LCM transfer film. The scattering media can be fabricated so as to shape the LCM beam and/or the illumination beam. The scattering media needs to be within a few millimeters of the sample to be effective. A few millimeters means less than one centimeter, preferably less than five millimeters.

The process of operating the instrument begins by visualizing the tissue from which the sample is to be acquired. The tissue is then moved to bring the portion that is to be acquired directly below the principal axis of the instrument. A laser capture microdissection transfer film is then set over the desired area. In a preferred embodiment the film is spaced to within a few microns of the top surface of the sample. Alternatively, the film can be placed in contact with the top of the sample with a pressure sufficient to allow transfer without forcing nonspecific transfer. Finally, the laser is

pulsed to heat the film and remove the tissue. The film needs to be pulled off of the sample quickly. Though the velocity should be such that the sample is thixotropically sheared.

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Practical Applications of the Invention

A practical application of the invention that has value within the technological arts is the collection of a large database of gene expression patterns of both healthy and diseased tissue, at different stages of diseases.
10 This database will be used to more fully understand that pathogenesis of cancer and infectious diseases. The invention will enable a scientist to identify gene patterns and incorporate this information into effective diagnostics for disease. The invention will allow medical doctors to compare actual patient tissue samples with archived data from patient samples at different disease stages, thereby allowing them to prescribe more effective stage therapies, eliminate unnecessary procedures, and reduce patient suffering. Other research areas where the invention will find use are drug discovery, developmental biology, forensics, botany, and the study of infectious diseases such a drug-resistant tuberculosis. There are virtually
15 innumerable uses for the invention, all of which need not be detailed here.
20

Advantages of the Invention

A laser capture microdissection instrument and/or method
25 representing an embodiment of the invention can be cost effective and advantageous for at least the following reasons. The invention will replace

current methods with better technology that allows for more accurate and reproducible results. The invention can be used to provide a low cost injection molded polymer disposable that integrates a laser capture microdissection transfer film into the interior surface of an analysis container such as a microcentrifuge tube (e.g., an EPPENDORF™ tube).

All publications, patent applications, and issued patents mentioned in this application are hereby incorporated herein by reference in their entirety to the same extent as if each individual publication, application, or patent was specifically and individually indicated to be incorporated in its entirety by reference.

All the disclosed embodiments of the invention described herein can be realized and practiced without undue experimentation. Although the best mode of carrying out the invention contemplated by the inventors is disclosed above, practice of the invention is not limited thereto. It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. Accordingly, it will be appreciated by those skilled in the art that the invention may be practiced otherwise than as specifically described herein.

For example, the individual components need not be formed in the disclosed shapes, or assembled in the disclosed configuration, but could be provided in virtually any shape, and assembled in virtually any configuration. Further, the individual components need not be fabricated from the disclosed materials, but could be fabricated from virtually any suitable materials. Further, although the LCM instrument disclosed herein is described as a physically separate module, it will be manifest that the

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LCM instrument may be integrated into other apparatus with which it is associated. Furthermore, all the disclosed elements and features of each disclosed embodiment can be combined with, or substituted for, the disclosed elements and features of every other disclosed embodiment except where such elements or features are mutually exclusive.

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It will be manifest that various additions, modifications and rearrangements of the features of the invention may be made without deviating from the spirit and scope of the underlying inventive concept. It is intended that the scope of the invention as defined by the appended claims and their equivalents cover all such additions, modifications, and rearrangements. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means-for." Expedient embodiments of the invention are differentiated by the appended subclaims.

CLAIMS

What is claimed is:

1. A laser capture microdissection method, comprising:
providing a sample that is to undergo laser capture microdissection;
positioning said sample within an optical axis of a laser capture
microdissection instrument, said laser capture microdissection instrument
including an illumination/laser beam delivery system;
providing a transfer film carrier having a substrate surface and a laser
capture microdissection transfer film coupled to said substrate surface;
placing said laser capture microdissection transfer film in
juxtaposition with said sample with a pressure sufficient to allow laser
capture microdissection transfer of a portion of said sample to said laser
capture microdissection transfer film, without forcing nonspecific transfer of
a remainder of said sample to said laser capture microdissection film; then
illuminating said sample with said illumination/laser beam delivery
system; and then
transferring a portion of said sample to said laser capture
microdissection transfer film, without forcing nonspecific transfer of a
remainder of said sample to said laser capture microdissection transfer film,
with said illumination/laser beam delivery system.
2. The method of claim 1, wherein said illumination/laser beam
delivery system includes a white light illuminator and the step of
illuminating said sample includes illuminating said sample with said white
light illuminator.

3. The method of claim 2, wherein illuminating said sample with said white light illuminator includes passing white light toward said transfer film carrier through both a dichroic mirror and a focusing lens.
4. The method of claim 3, further comprising superimposing a beam from a laser with white light illumination from said white light illuminator.
5. The method of claim 1, wherein said illumination/laser beam delivery system includes a laser capture microdissection optical train and the step of transferring a portion of said sample includes transferring a portion of said sample with said laser capture microdissection optical train.
6. The method of claim 5, wherein transferring a portion of said sample with said laser capture microdissection optical train includes reflecting a collimated beam with a beam steering mirror and then reflecting said collimated beam with a dichroic mirror through a focusing lens toward said transfer film carrier.
7. The method of claim 6, further comprising adjusting a beam spot size with said focusing lens, said beam spot size being defined by said collimated beam.
8. The method of claim 6, further comprising changing a beam diameter with a variable aperture, said beam diameter being defined by said collimated beam.

9. The method of claim 6, further comprising passing said collimated beam through an objective and then reflecting said collimated beam to a cut-off filter.
10. The method of claim 5, further comprising superimposing a beam from said laser capture microdissection optical train with white light illumination from a white light illuminator.
11. The method of claim 1, further comprising delivering optical information to an image acquisition system with said illumination/laser beam delivery system.
12. The method of claim 1, further comprising delivering optical information to an eyepiece assembly with said illumination/laser beam delivery system.
13. The method of claim 1, wherein said sample includes a fluorescent system, and, further comprising exciting said fluorescent system.
14. The method of claim 13, further comprising identifying at least a portion of said sample with light that excites said fluorescent system, before the step of transferring.
15. The method of claim 1, wherein illuminating said sample includes condensing a collimated beam of illumination light.

16. The method of claim 15, wherein illuminating said sample includes passing said collimated beam of illumination light through a beam splitter.
17. The method of claim 16, wherein transferring said portion of said sample includes injecting a laser beam by reflecting said laser beam with said beam splitter.
18. The method of claim 15, wherein said collimated beam of illumination light is obtained by collimating a diverging beam of illumination light with an aspheric lens.
19. The method of claim 18, wherein said diverging beam of illumination light is obtained from a fiber optic.
20. The method of claim 1, wherein the step of illuminating said sample includes scattering illumination light with a scattering media.
21. The method of claim 20, wherein scattering illumination light with said scattering media includes scattering illumination light with said transfer film carrier.
22. A laser capture microdissection instrument, comprising:
an illumination/laser beam delivery system.

23. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a white light illuminator.
24. The laser capture microdissection instrument of claim 23, wherein said illumination/laser beam delivery system includes a dichroic mirror optically coupled to said while light illuminator and a focusing lens optically coupled to said dichroic mirror.
25. The laser capture microdissection instrument of claim 24, wherein said illumination/laser beam delivery system includes a laser diode optically coupled to said focusing lens.
26. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a laser capture microdissection optical train.
27. The laser capture microdissection instrument of claim 26, wherein said laser capture microdissection optical train includes a laser, a beam steering mirror optically coupled to said laser, a dichroic mirror optically coupled to said beam steering mirror, and a focusing lens optically coupled to said dichroic mirror.
28. The laser capture microdissection instrument of claim 27, wherein said laser capture microdissection optical train includes an objective

optically coupled to said focusing lens and a cut-off filter optically coupled to said objective.

29. The laser capture microdissection instrument of claim 27, wherein said laser capture microdissection optical train includes a variable aperture optically coupled to said focusing lens.

30. The laser capture microdissection instrument of claim 27, wherein said laser capture microdissection optical train includes a stepped prism that can be optically coupled to said focusing lens.

31. The laser capture microdissection instrument of claim 27, wherein said illumination/laser beam delivery system includes a white light illuminator optically coupled to said dichroic mirror.

32. The laser capture microdissection instrument of claim 22, further comprising an image acquisition system optically coupled to said illumination/laser beam delivery system.

33. The laser capture microdissection instrument of claim 22, further comprising an eyepiece assembly optically coupled to said illumination/laser beam delivery system.

34. The laser capture microdissection instrument of claim 22, further comprising a fluorescent system optically coupled to said illumination/laser beam delivery system.

35. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a condensing lens.
36. The laser capture microdissection instrument of claim 35, wherein said illumination/laser beam delivery system includes a beam splitter optically coupled to said condensing lens.
37. The laser capture microdissection instrument of claim 36, wherein said illumination/laser beam delivery system includes a laser optically coupled to said beam splitter.
38. The laser capture microdissection instrument of claim 37, wherein said illumination/laser beam delivery system includes a fiber optic optically coupled to said beam splitter.
39. The laser capture microdissection instrument of claim 22, wherein said illumination/laser beam delivery system includes a scattering media.
40. The laser capture microdissection instrument of claim 39, wherein said scattering media includes a transfer film carrier.
41. The laser capture microdissection instrument of claim 22, further comprising a translation stage coupled to said illumination/laser beam delivery system.

42. The laser capture microdissection instrument of claim 41, further comprising a manual joystick subsystem connected to said translation stage.
43. The laser capture microdissection instrument of claim 41, further comprising a vacuum chuck subsystem connected to said translation stage.
44. The laser capture microdissection instrument of claim 22, further comprising a transfer film carrier handling subsystem coupled to said illumination/laser beam delivery system.
45. An inverted microscope, comprising:
an illumination/laser beam delivery system.
46. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a white light illuminator.
47. The inverted microscope of claim 46, wherein said illumination/laser beam delivery system includes a dichroic mirror optically coupled to said white light illuminator and a focusing lens optically coupled to said dichroic mirror.
48. The inverted microscope of claim 47, wherein said illumination/laser beam delivery system includes a laser diode optically coupled to said focusing lens.

49. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a laser capture microdissection optical train.
50. The inverted microscope of claim 49, wherein said laser capture microdissection optical train includes a laser, a beam steering mirror optically coupled to said laser, a dichroic mirror optically coupled to said beam steering mirror, and a focusing lens optically coupled to said dichroic mirror.
51. The inverted microscope of claim 50, wherein said laser capture microdissection optical train includes an objective optically coupled to said focusing lens and a cut-off filter optically coupled to said objective.
52. The inverted microscope of claim 50, wherein said laser capture microdissection optical train includes a variable aperture optically coupled to said focusing lens.
53. The inverted microscope of claim 50, wherein said laser capture microdissection optical train includes a stepped prism that can be optically coupled to said focusing lens.
54. The inverted microscope of claim 50, wherein said illumination/laser beam delivery system includes a white light illuminator optically coupled to said dichroic mirror.

55. The inverted microscope of claim 45, further comprising an image acquisition system optically coupled to said illumination/laser beam delivery system.
56. The inverted microscope of claim 45, further comprising an eyepiece assembly optically coupled to said illumination/laser beam delivery system.
57. The inverted microscope of claim 45, further comprising a fluorescent system optically coupled to said illumination/laser beam delivery system.
58. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a condensing lens.
59. The inverted microscope of claim 58, wherein said illumination/laser beam delivery system includes a beam splitter optically coupled to said condensing lens.
60. The inverted microscope of claim 59, wherein said illumination/laser beam delivery system includes a laser optically coupled to said beam splitter.
61. The inverted microscope of claim 60, wherein said illumination/laser beam delivery system includes a fiber optic optically coupled to said beam splitter.

62. The inverted microscope of claim 45, wherein said illumination/laser beam delivery system includes a scattering media.
63. The inverted microscope of claim 62, wherein said scattering media includes a transfer film carrier.
64. The inverted microscope of claim 45, further comprising a translation stage coupled to said illumination/laser beam delivery system.
65. The inverted microscope of claim 64, further comprising a manual joystick subsystem connected to said translation stage.
66. The inverted microscope of claim 64, further comprising a vacuum chuck subsystem connected to said translation stage.
67. The inverted microscope of claim 45, further comprising a transfer film carrier handling subsystem coupled to said illumination/laser beam delivery system.

ABSTRACT OF THE DISCLOSURE

Systems and methods for laser capture microdissection are disclosed. An inverted microscope includes an illumination/laser beam delivery system that is adapted to both illuminate a sample and provide energy for laser capture microdissection of the sample. The systems and methods provide the advantages of increased speed and much lower rates of contamination.

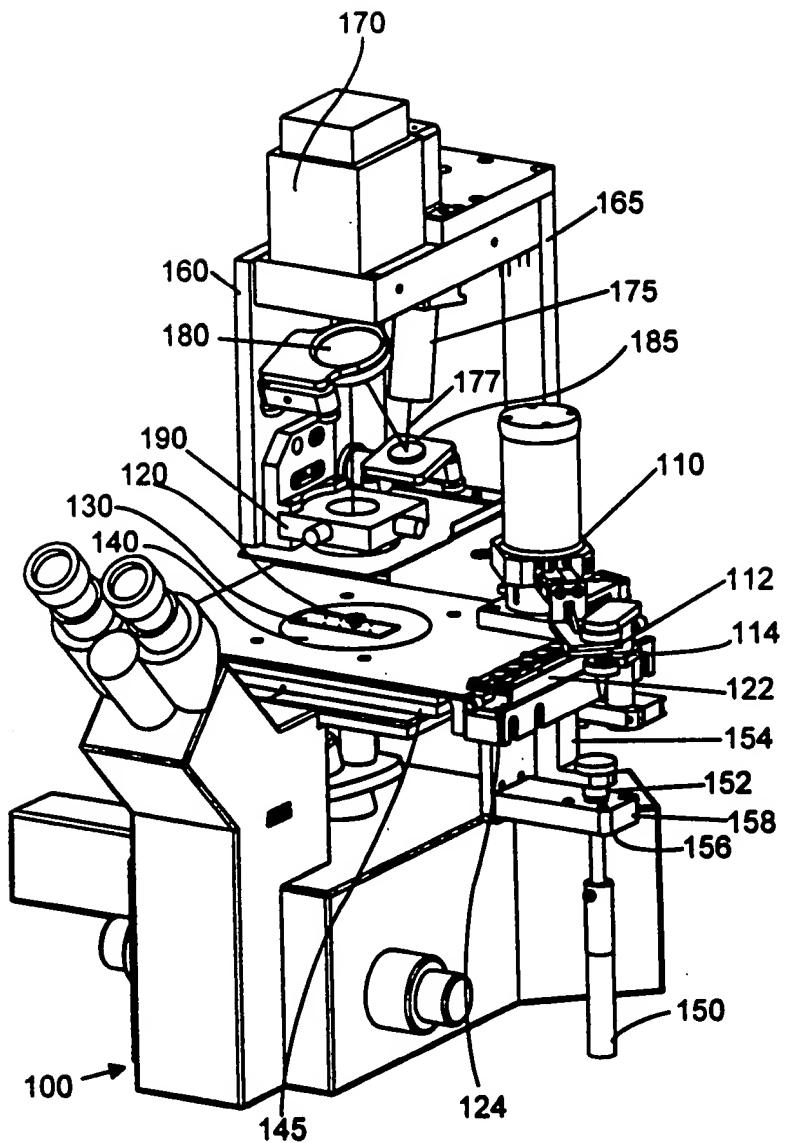
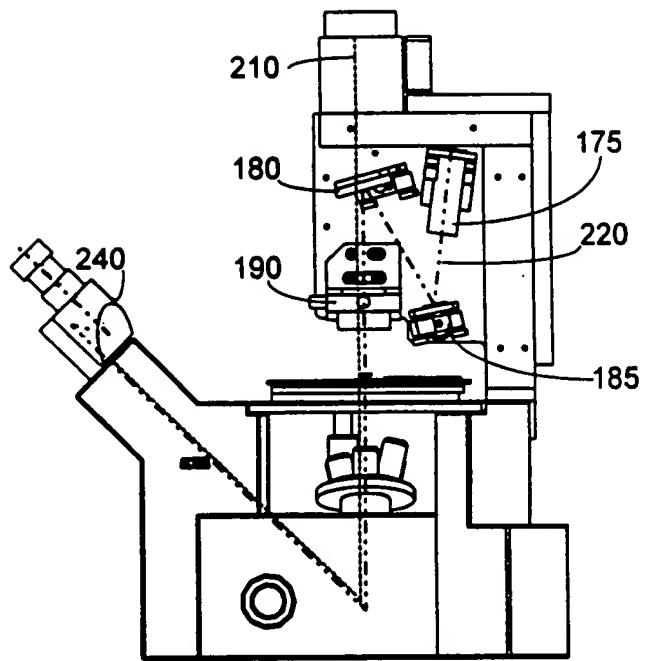
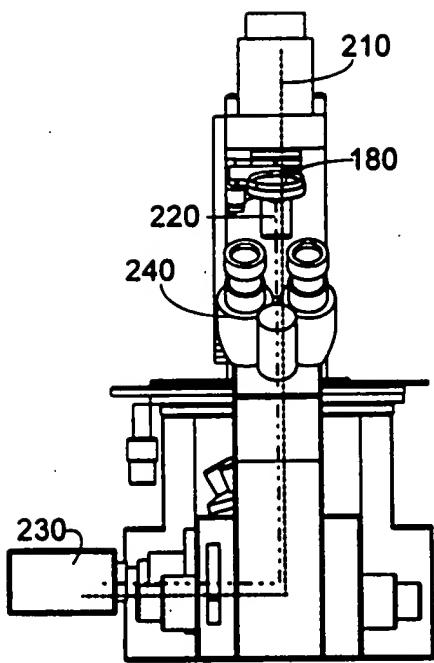


FIG. 1



— WHITE LIGHT ILLUMINATION PATH
— LASER BEAM PATH

FIG. 2A

FIG. 2B

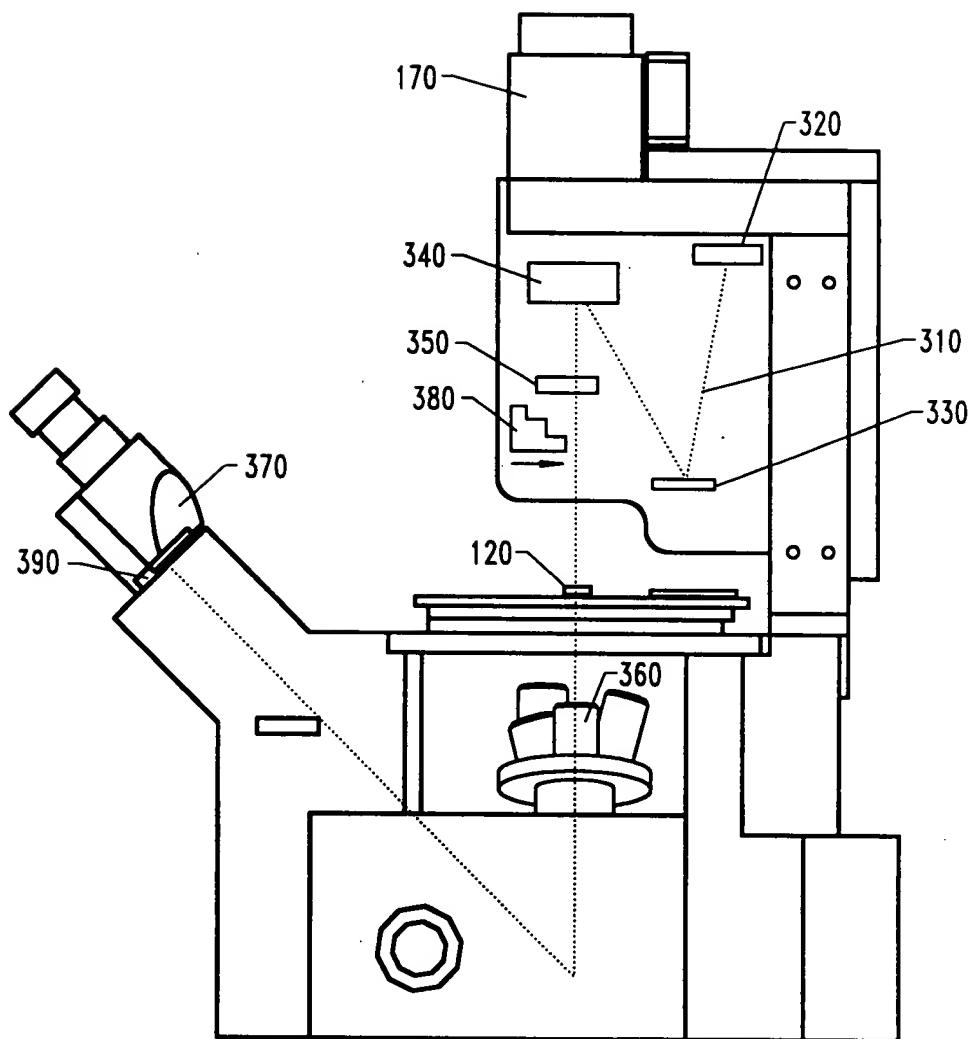


FIG.3

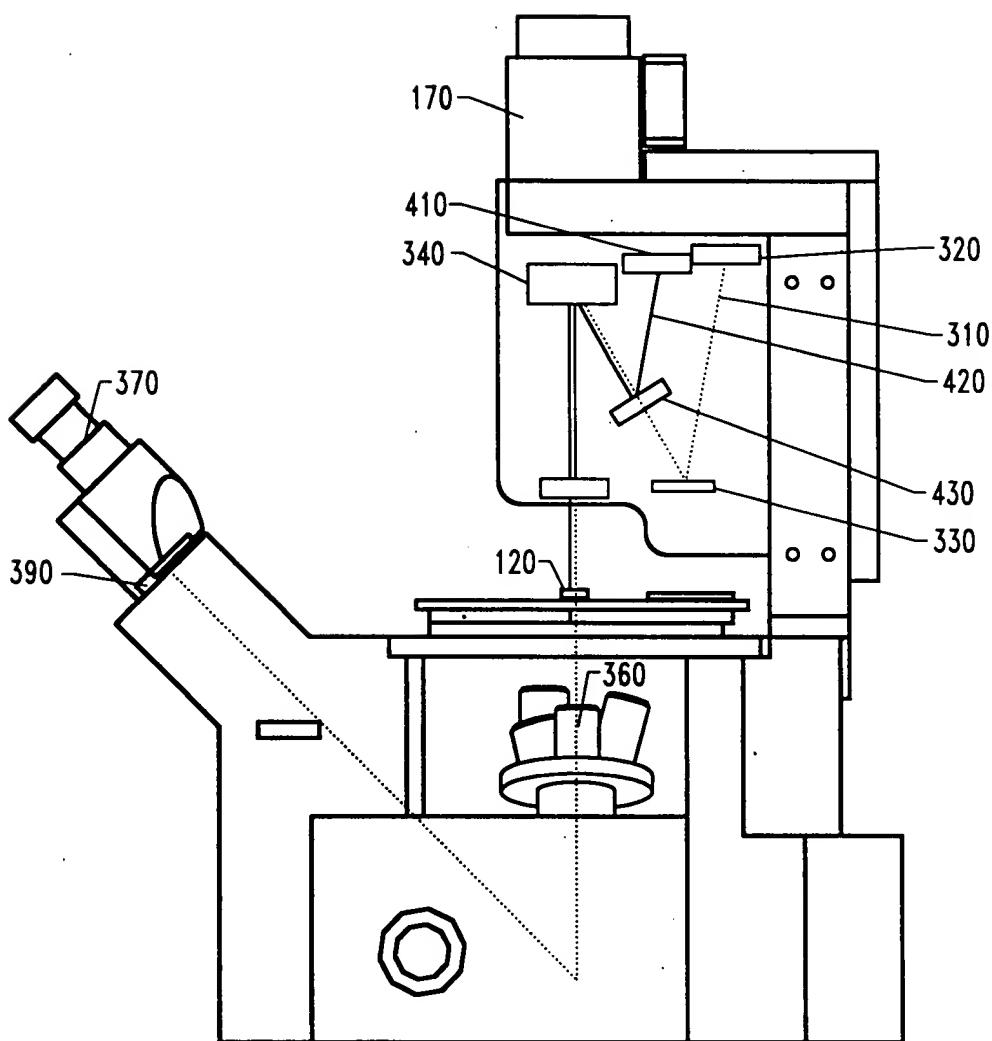


FIG. 4

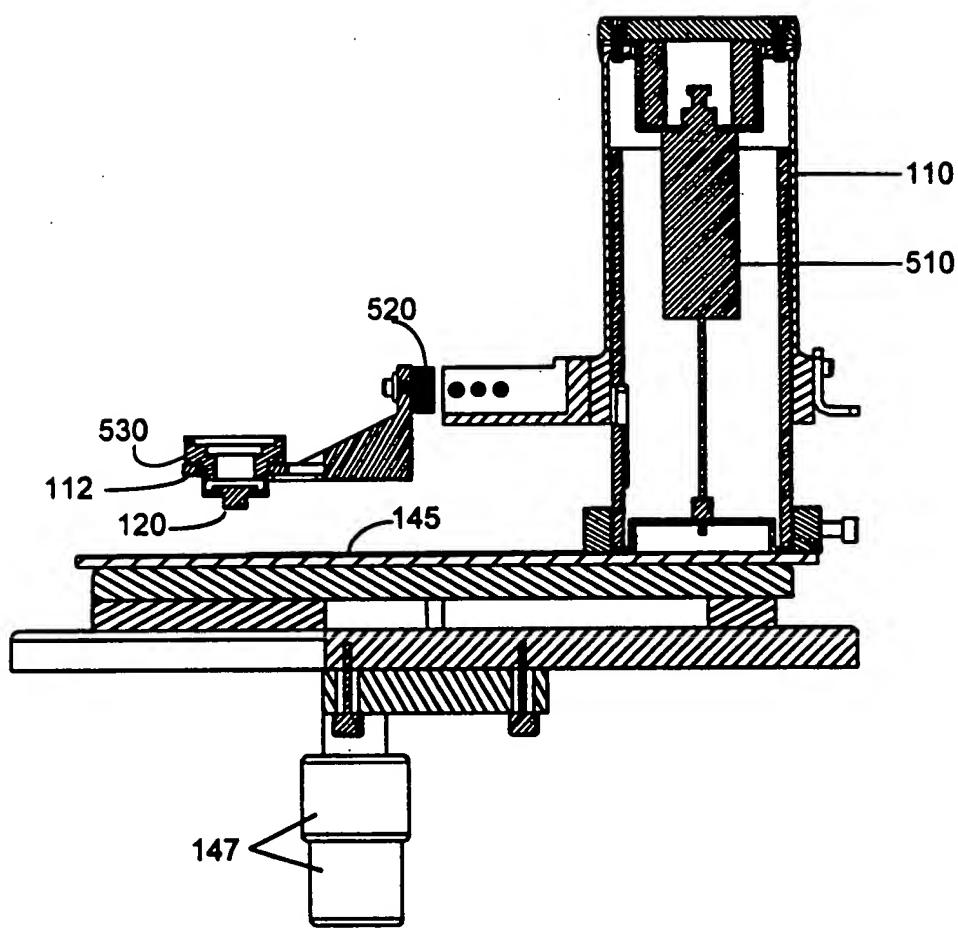


FIG. 5

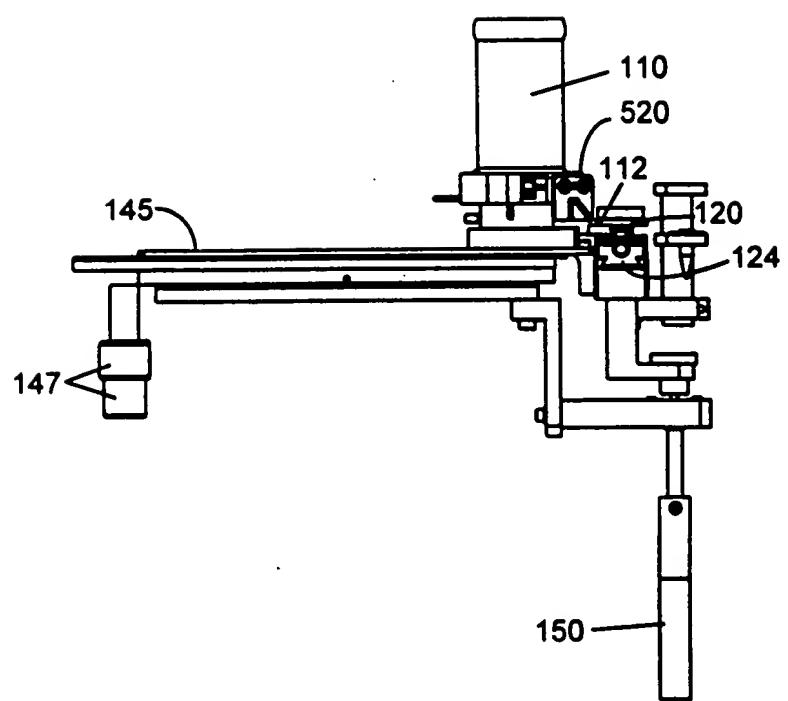


FIG. 6

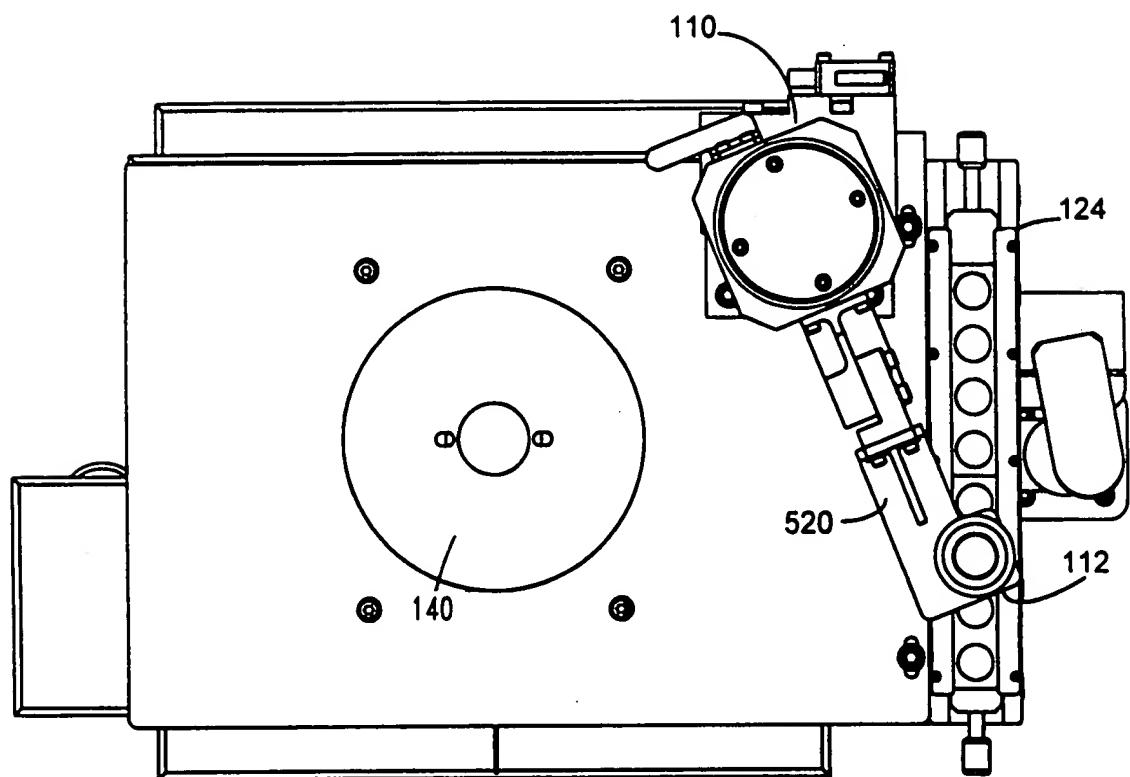


FIG. 7

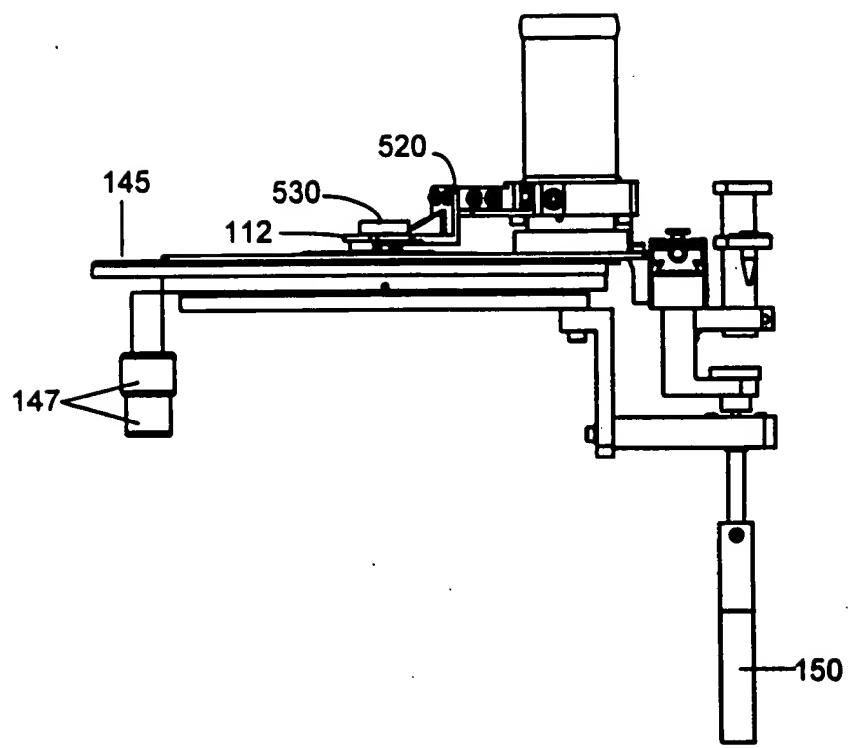


FIG. 8

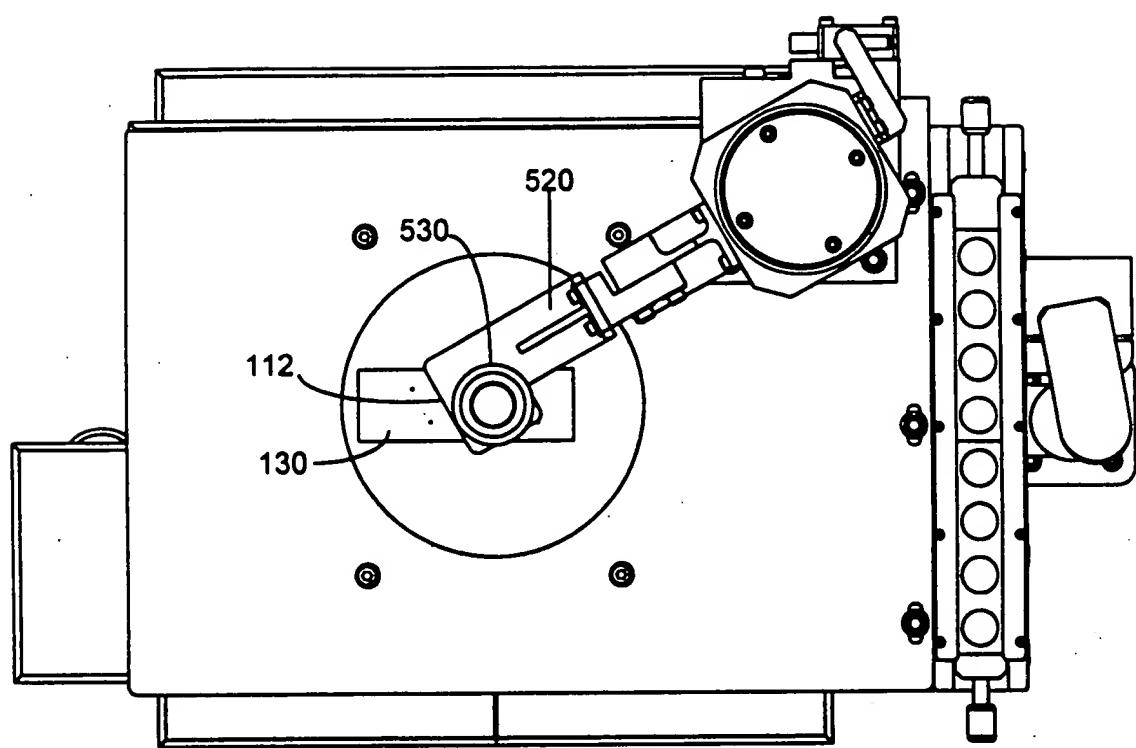


FIG. 9

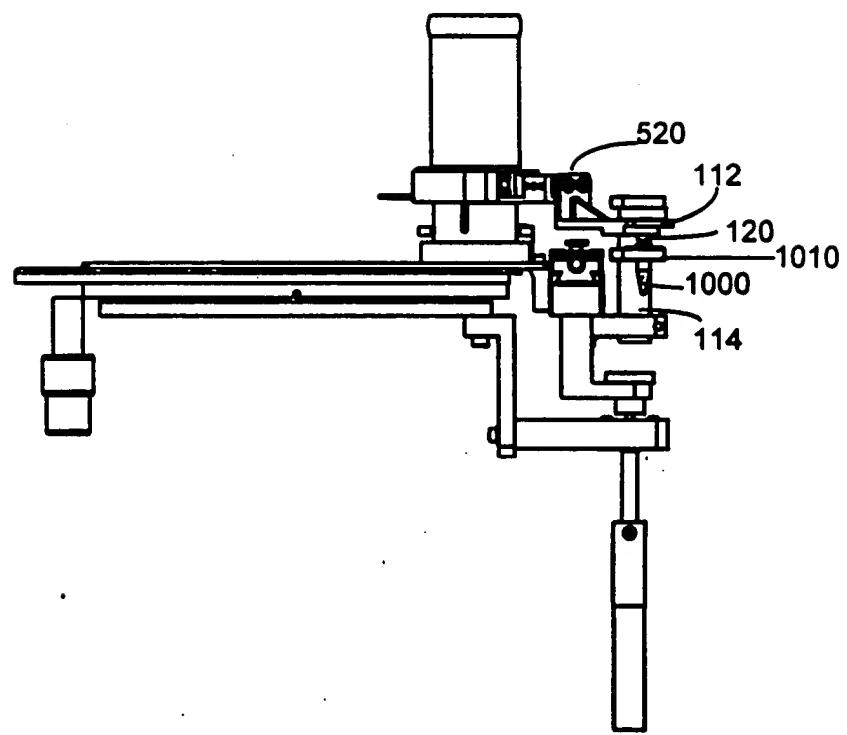


FIG. 10

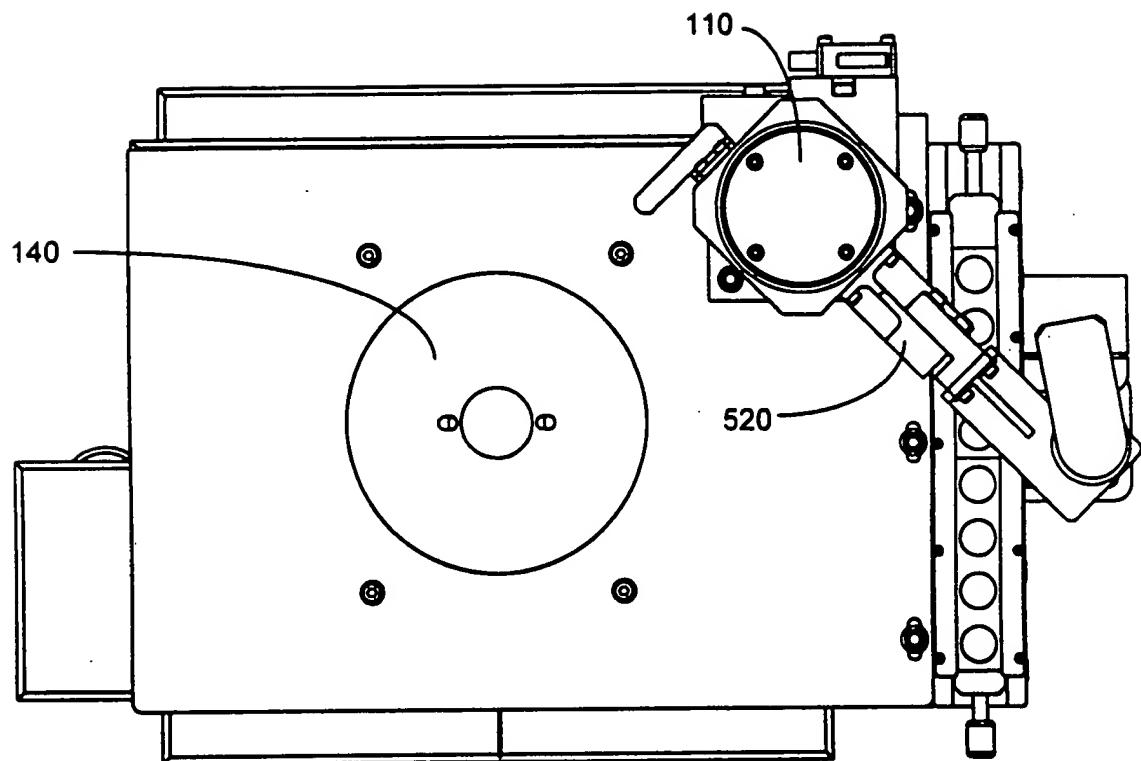


FIG. 11

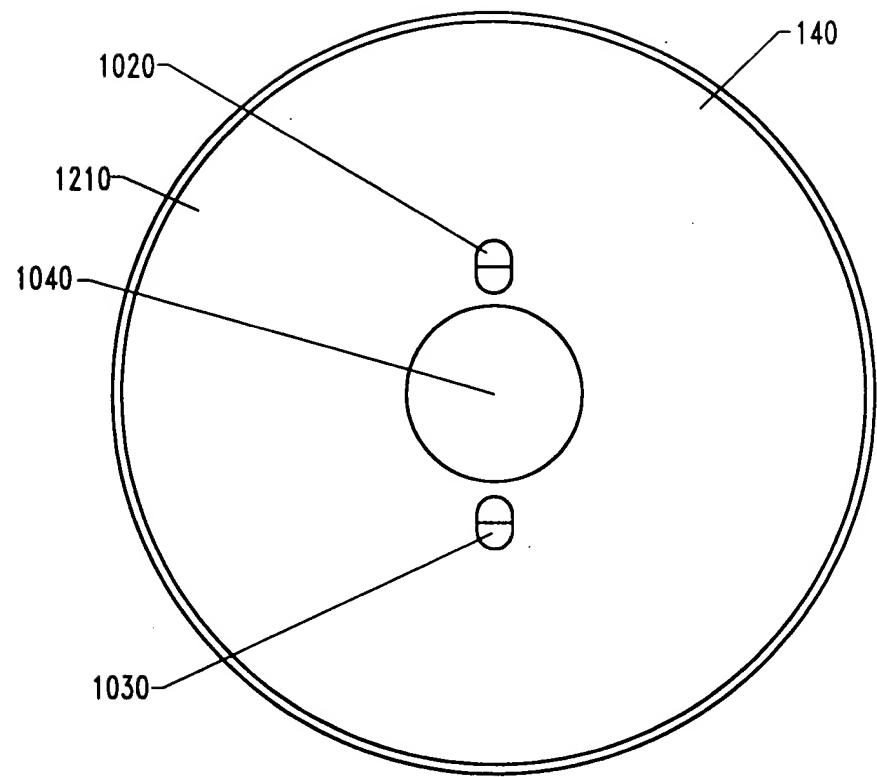


FIG. 12

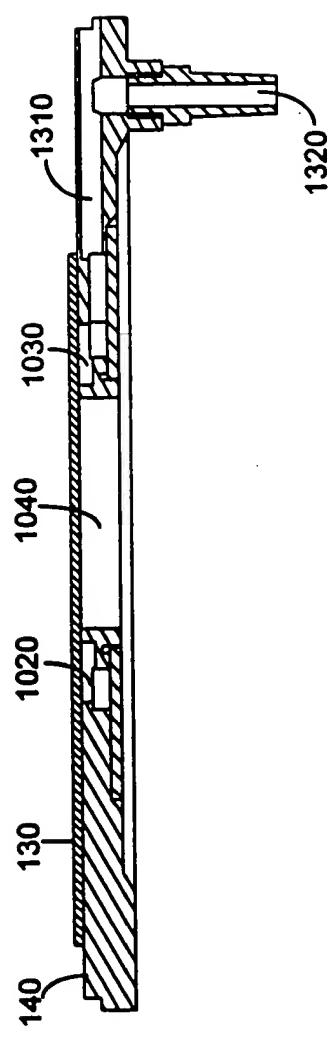


FIG. 13

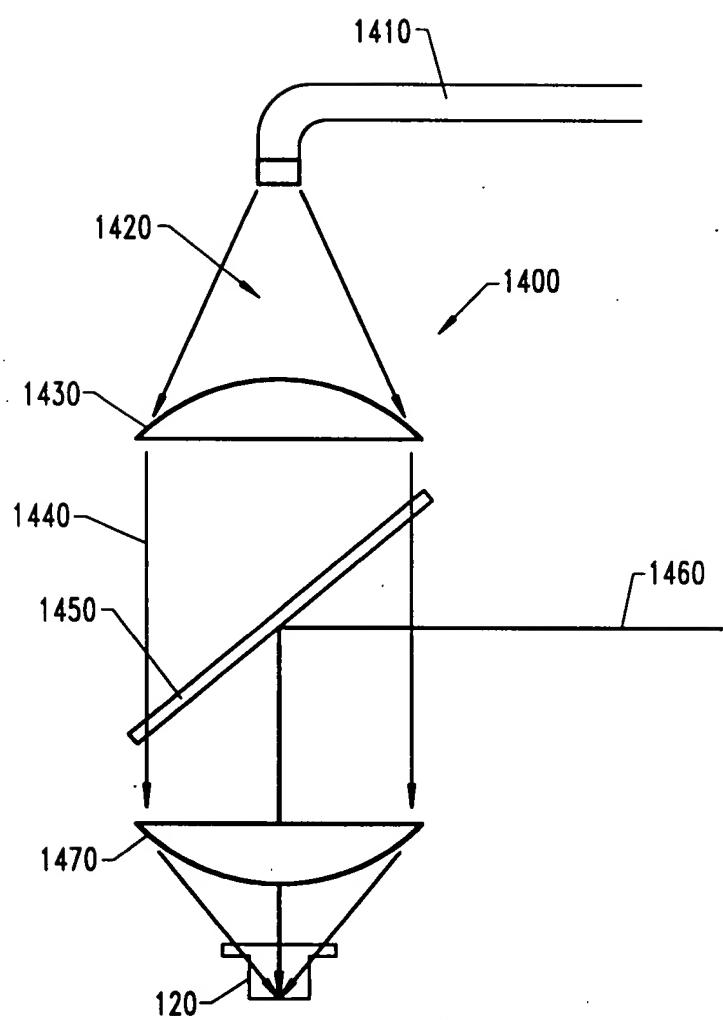


FIG. 14

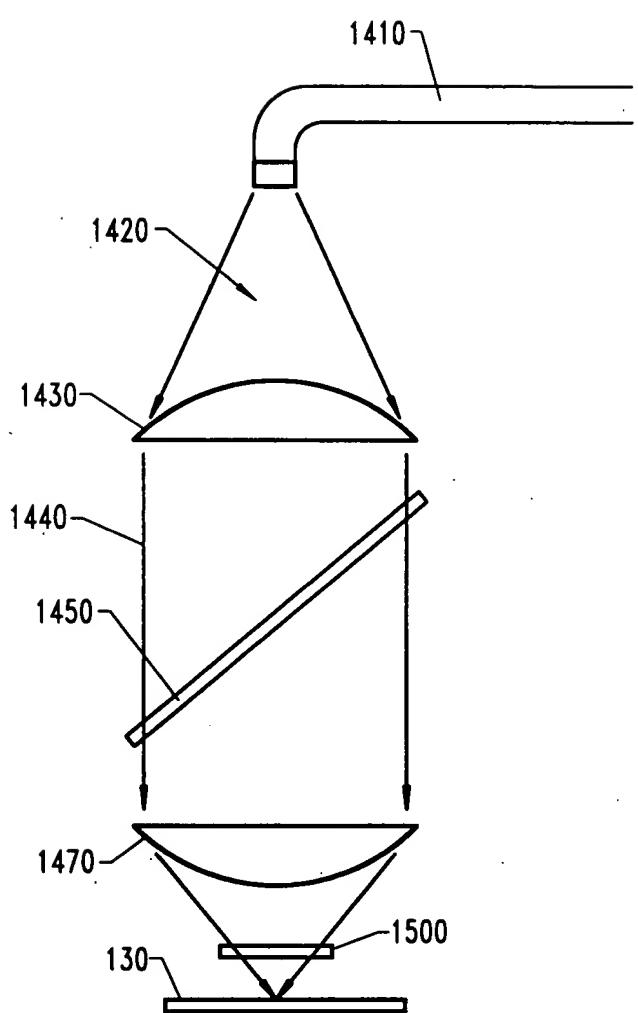


FIG. 15

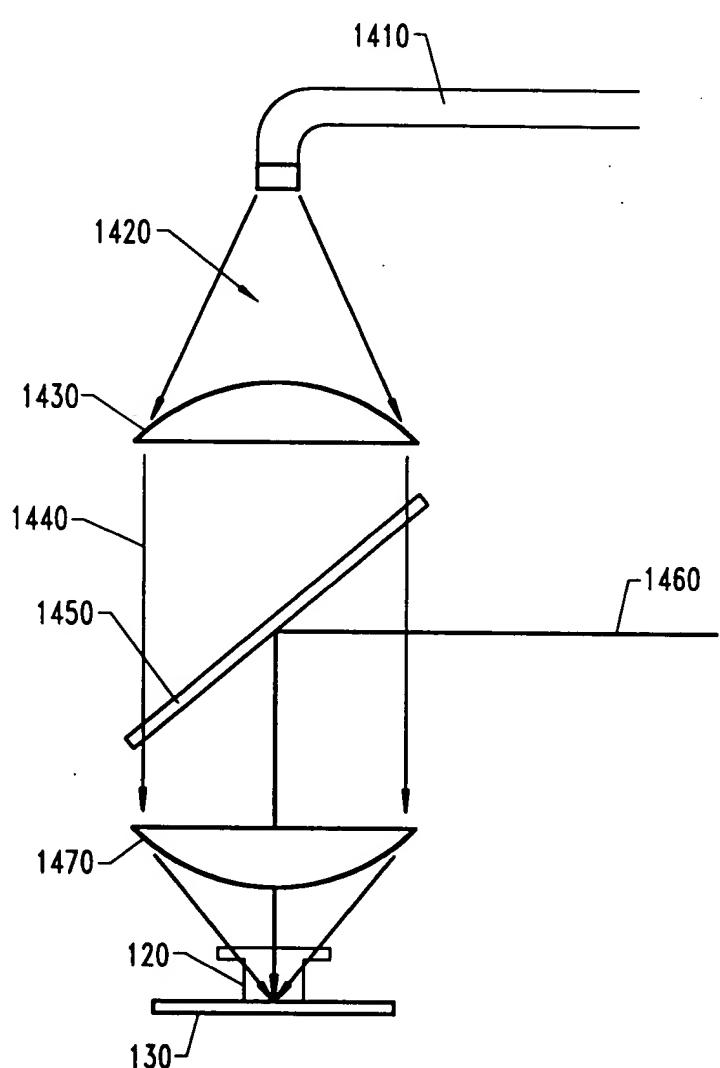


FIG. 16

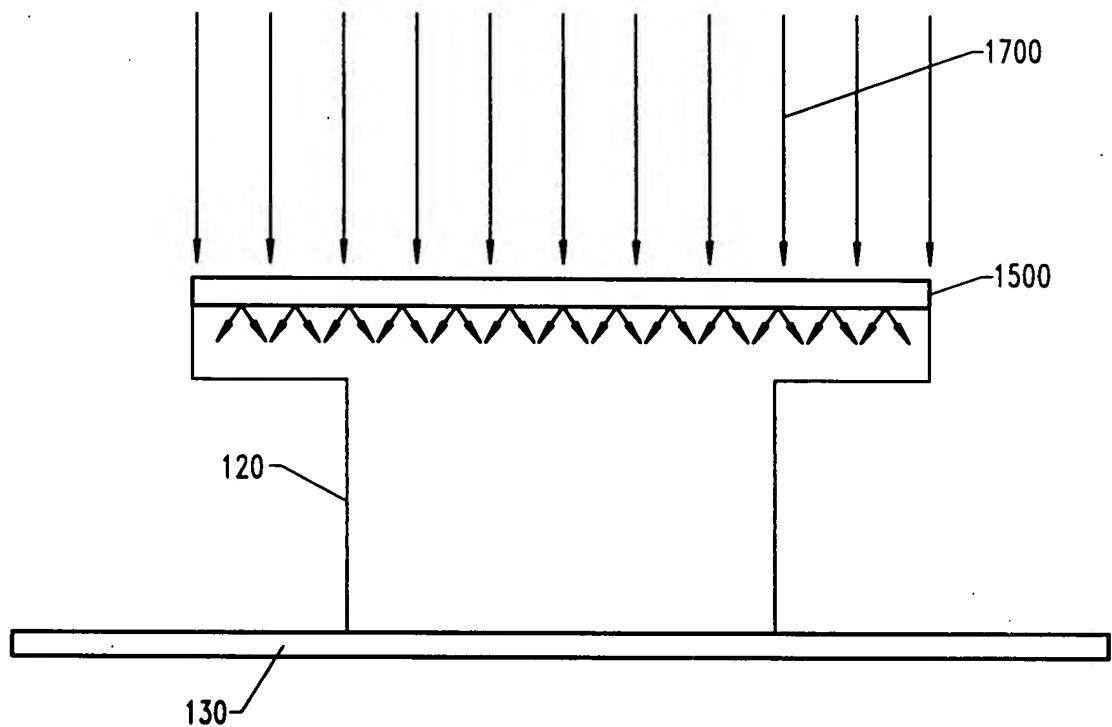


FIG. 17

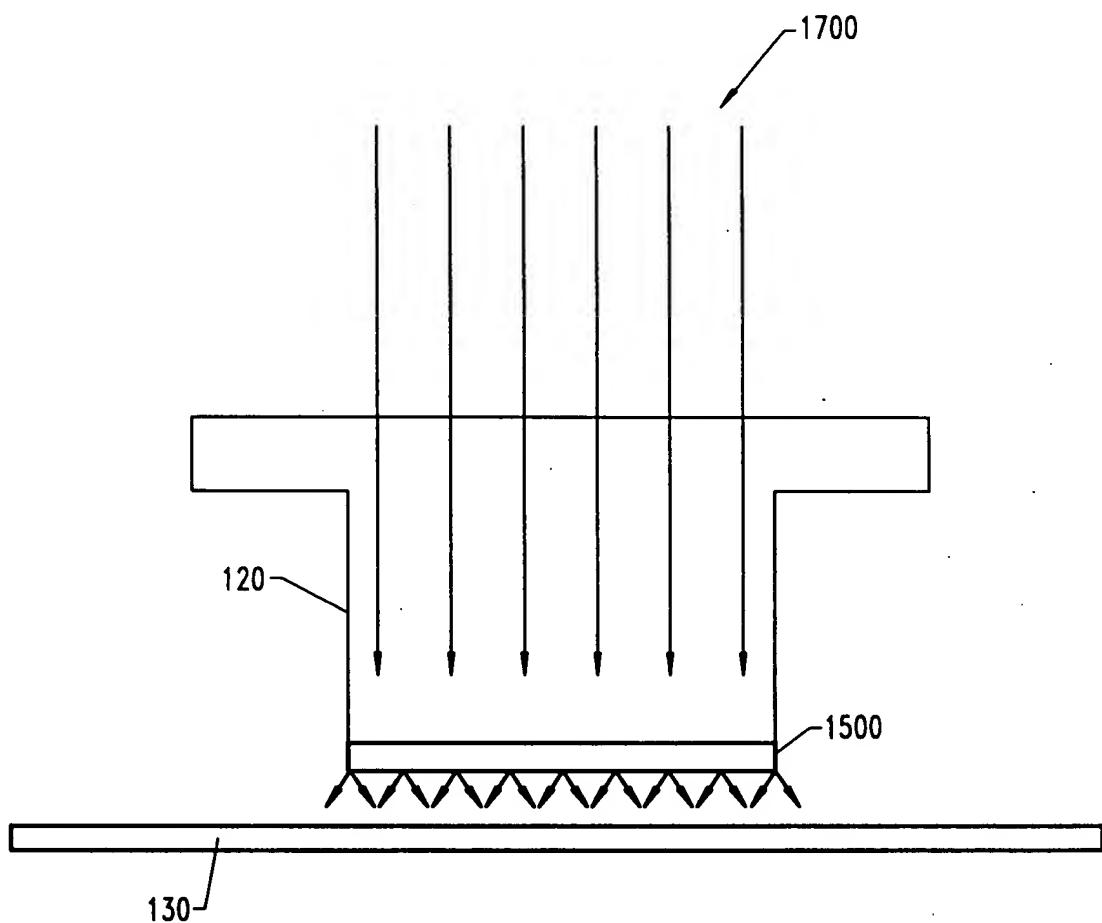


FIG. 18